SOIL Discuss., https://doi.org/10.5194/soil-2018-7
Manuscript under review for journal SOIL
Discussion started: 16 May 2018

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

Thomas Behrendt[1,†], Elisa C. P. Catão[1], Rüdiger Bunk[2], Zhigang Yi[2,3], Elena Schwer[1], Jürgen Kesselmeier[2], Susan Trumbore[1]

[1]Department Biogeochemical Processes, Max Planck Institute for Biogeochemistry, Jena

[2]Department Multiphase Chemistry, Max Planck Institute for Chemistry, Mainz

[3]College of Resources and Environment, Fujian Agriculture and Forestry University, Fuzhou, China

†Correspondence to: tbehr@bgc-jena.mpg.de, Phone: +49-(0) 3641-57-6105, Fax: +49-(0) 3641-57-70

Keywords: carbonyl sulfide production, thiocyanate degradation, carbonyl sulfide consumption, nitric oxide, carbon monoxide, CO_2 fixation

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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 (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]. Several different soils were rewetted and soil-air exchange monitored as soils dried out to investigate responses to changing moisture levels. A major control of OCS exchange is the total amount of available S in the soil. P_{OCS} production rates were highest for soils at WFPS > 60 % and rates were negatively related to thiosulfate concentrations. These soils flipped from being net sources to net sinks of OCS at moderate moisture levels (WFPS 15 to 37 %). By measuring CO and NO while fumigating soils at different levels of OCS, we could show that CO consumption and NO exchange are linked to U_{OCS} under moderate soil moisture. Based on the OCS:CO flux ratio two different U_{OCS} processes could be separated. For one of the investigated soils, we demonstrated changes in microbial activity 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.

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1 Introduction

Carbonyl sulfide (OCS) is the most abundant sulfur containing trace gas in the troposphere with a life time on the order of years. OCS contributes to warming the troposphere and cooling in the stratosphere, both processes are considered balanced (Brühl et al., 2012). Plants simultaneously take up carbon dioxide (CO_2) and OCS by contribution of the enzymes Ribulose-1,5-Bisphosphate-Carboxylase (RubisCO) and Phosphoenolpyruvate-Carboxylase (PEPCO), enhanced by Carbonic anhydrase (CA, Protoschill-Krebs and Kesselmeier, 1992; Protoschill-Krebs et al. 1996). Thus, fluxes of OCS are closely related to gross photosynthesis, and represent the largest global OCS sink with 0.73-1.5 Tg S a^{-1} (Sandoval-Soto et al., 2005).

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), upland soils are accounted as a sink for OCS of about 0.355 Tg a^{-1} (Berry et al., 2013). OCS uptake in soils is thought to be dominated by CA (Wingate et al., 2009), but there is evidence that RubisCO might also play a role (Whelan et al., 2017; Kesselmeier et al., 1999). The microbial mechanisms underlying OCS production (P_{OCS}) and consumption (U_{OCS}), however, are not yet known (Ogée et al., 2016). In fact, current studies report that soils can flip 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, better understanding of environmental factors interacting with the soil microbial community is required for predicting net soil OCS fluxes from the ecosystem to global scale.

The majority of OCS is 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). 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 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 associated with utilization of either CO_2 or bicarbonate (HCO_3^-) substrates by carboxylases. These enzymes can be differentiated and are similar to those found in plants (Erb, 2011). Carbonic 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, it is not only present in soils and higher plants but also in algae and lichens, 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 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).

RubisCO is present in all phototrophic tissues and cells and some autotrophic microorganisms in soils (Badger and Bek, 2008), and thus is also a candidate for OCS consumption. In 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, elevated CO_2 levels have been discussed to have the potential for competitive inhibition of RubisCO but not CA or PEPCO

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(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, could degrade OCS. Initial measurements of sulfur isotopic fractionation factors ($^{34}\varepsilon$) (Kamezaki et al., 2016) indicate the potential to estimate the OCS sink in soils using $\delta^{34}S$ measurements.

Additional clues to processes controlling uptake of OCS in soils come from observing relationships with other gases consumed by soils, such as CO. There is evidence that the sinks for CO and OCS are related to each other: (1) Nitrifiers and methanotrophs are capable of aerobic CO co-oxidation via ammonia monooxygenase (AMO) and methane monooxygenase (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). The energy 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) acetogens, methanogens, and sulfate reducers are able to catalyze the oxidation of CO via carbon monoxide dehydrogenase (CODH) anaerobically within the Wood-Ljungdahl pathway 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 affinity for the substrate OCS, expressed as K_M, is about 2.2 mM 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). Thus, it is

assumed that the activity of different enzymes is expressed in the OCS:CO ratio. Sun and co-workers (2017) showed that OCS:CO_2 ratios are related to CO:CO_2 ratios in a boreal forest. Abiotic CO production, which is dependent on the temperature of photodecomposition of organic matter (Conrad & Seiler, 1985), occurs also in soils, but under dark incubation are expected to be small.

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 1×10^{-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 inactivation of RubisCO. It can be hypothesized that the substrate affinity of RubisCO for CO_2 and OCS differs (see Lorimer and Pierce 1989).

Further 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 9 samples of topsoil (integrating a depth between 0-5 cm) were used, representing different soil types and land uses. To make a representative sample for each site, 9 individual samples 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.

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), soil (~ 60 g) was moistened to field capacity (100 % water-filled pore space, WFPS) for most soils; 80 % WFPS for 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 (~ 400 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 samples 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. 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 production and consumption contributions to the net flux (Behrendt et al., 2014). 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 field 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 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 gases is measured according the Bouguer Lambert Beer's law. For incubations of A1 and 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). 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, CO, and NO 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. The fluxes were calculated for 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). Biom 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 under ambient (500 ppt) OCS

After wetting stored soils to 80-100 % WFPS (field capacity), all agricultural soils except the sugar beet soil (A3) produced OCS, with rates of production declining as the soil dried out. At ~ 37 % $WFPS_{lab}$, these soils 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 soils, A1 and A2, produced the most OCS, up to 2 and 13 pmol g^{-1} h^{-1}, respectively. For the sugar beet soil, OCS fluxes were < 1.09 pmol g^{-1} h^{-1} (undetectable) or negative (net 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 A1. The grassland soil A5 produced up to 5 pmol g^{-1} h^{-1} OCS and was the only A soil that emitted OCS > 1.09 pmol g^{-1} h^{-1} within the range of moderate soil moisture. Both, 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, 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 A3 (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).

While A and F soils showed similar patterns that included a second increase in OCS production at about 10 % $WFPS_{lab}$, D soils only produced OCS. The different behavior for OCS exchange from desert soils may be related to differences in soil properties: 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 %). However, 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 fungi involved in P_{OCS} and U_{OCS} from A1 soil under different OCS fumigation (sequencing)

Overall, the sequencing approach did not result in significant differences in 16S rRNA transcript relative abundance for bacterial classes for the A1 soil fumigated at 50 versus 1000 ppt OCS at moderate soil moisture (Fig. 3a). In contrast, for ITS transcripts the relative abundance of Ascomycota (p-value = 0.006) 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 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.

3.3 Effect of OCS fumigation on CO exchange

For the 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 (qPCR) and NO exchange

The change in 16S rRNA transcript relative abundance for bacteria (sequencing) could not resolve significant differences for 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* transcripts. For the air dried A1 sample, 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.

SOIL Discuss., https://doi.org/10.5194/soil-2018-7
Manuscript under review for journal SOIL
Discussion started: 16 May 2018

4 Discussion

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

OCS is produced by the degradation of various S compounds. Thiocyanate 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).

In all soils, 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.

4.2 The role of bacteria and fungi involved in P_{OCS} and U_{OCS} from 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, since pure cultures from strains of *Umbelopsis*/*Mortierella* sp. were net producers of OCS. 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). 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 amplifier" for e.g. RubisCO and PepCO. Due to similarity of the OCS and CO_2 molecules, it seems reasonable that in chemical pathways of OCS consumption the roles of RubisCO and PepCO were

underestimated. 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 CO exchange – similarity to N_2O:NO ratio?

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 reanalyse 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 for 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 (qPCR) and NO exchange

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 2.57×10^{-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 accepted as dominant OCS consumers in literature, 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 (Bunk et al., submitted; Sauze et al., 2017; 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. 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.

Acknowledgements

The work has been funded by the Max Planck Society and Deutsche Forschungsgemeinschaft (DFG) CRC 1076 “AquaDiva”. We thank Alexander Moravek, Dianming Wu, and Moawad Badawy for support in collecting the soil samples.

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Tab. 1 Summary of soil samples and experimental conditions used for analysis. Note that OCS fluxes for F3, F4, F5 and A1 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.

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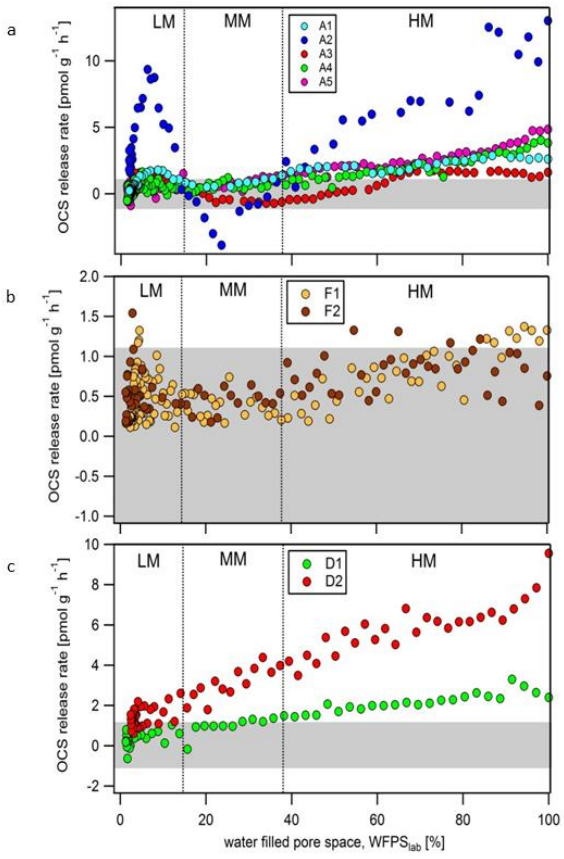

Fig. 1 OCS exchange rates from soil samples originated from agriculture (**a**) A1 to A5: cornfield (light blue), cornfield (dark blue), sugar beet (red dots), wheatfield (green), and grassland (pink), (**b**) F1, F2: natural rainforest (orange) and annual burned rainforest (brown), and (**c**) D1, D2: 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.

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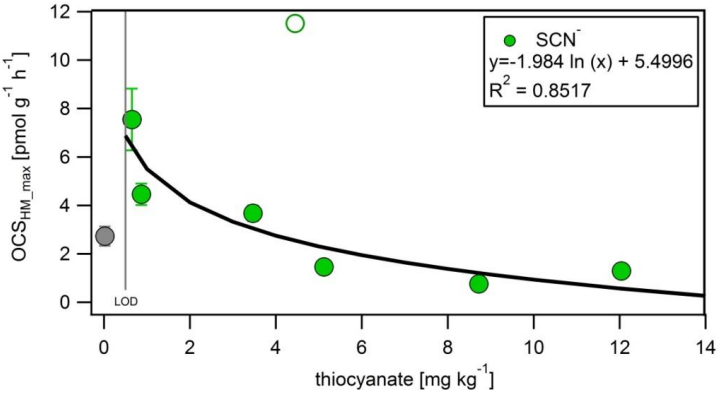

Fig. 2 Correlation between OCS exchange rate, $OCS_{max,HM}$ and thiocyanate (SCN^-) at high soil moisture for samples F1, F2, A3, A4, A5 (green). The maximum OCS exchange rate and thiocyanate concentration for A2 (green circle) are considered as an outlier, possibly due to release of thiocyanate from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

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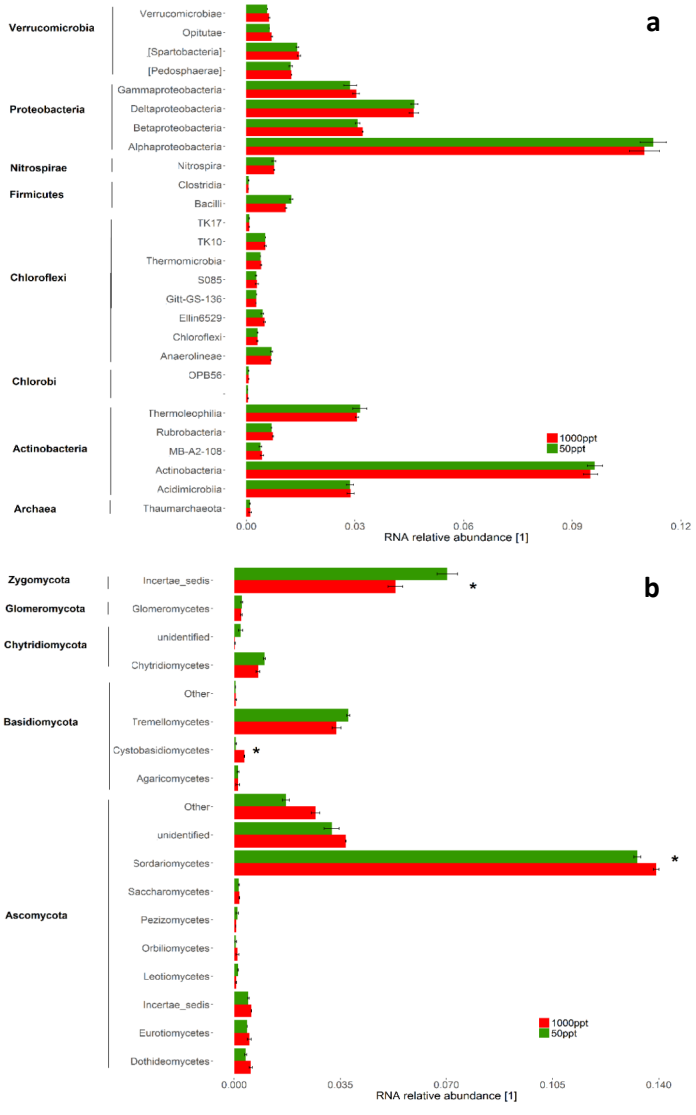

Fig. 3 Taxonomic composition of the mid-latitude corn field soil Mainz, Germany, at 22 % $WFPS_{lab}$ of the samples under 1000 ppt or 50 ppt OCS. Relative abundance of (a) 16S rRNA transcripts for selected bacterial classes and (b) internal transcribed spacer (ITS) transcripts for fungal classes, normalized by the total number of assigned reads per sample. Classes with RNA relative abundance < 5 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). 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 %).

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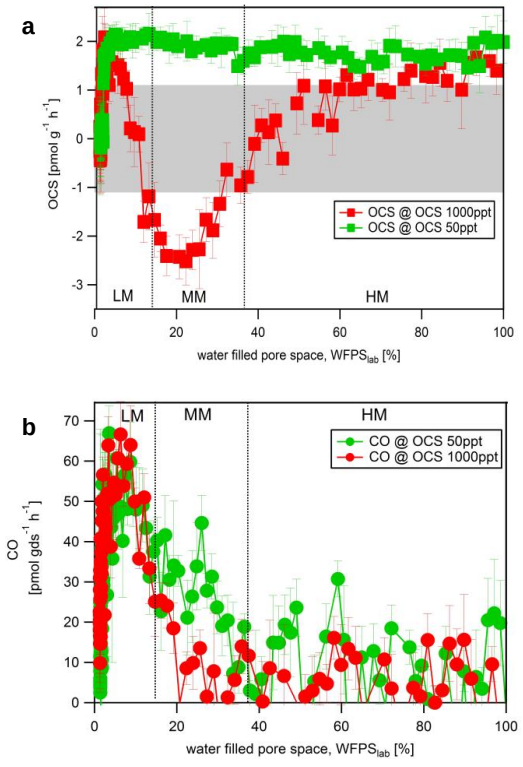

Fig. 4 OCS exchange rates (a) and CO exchange rates (b) at OCS mixing ratio of 50 ppt (green) and 1000 ppt (red) are shown for the A1 soil sample from a mid-latitude corn field, Mainz, Germany, data for (a) adapted from Bunk et al., submitted. Grey shaded area represents threshold 1.09 to -1.09 pmol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

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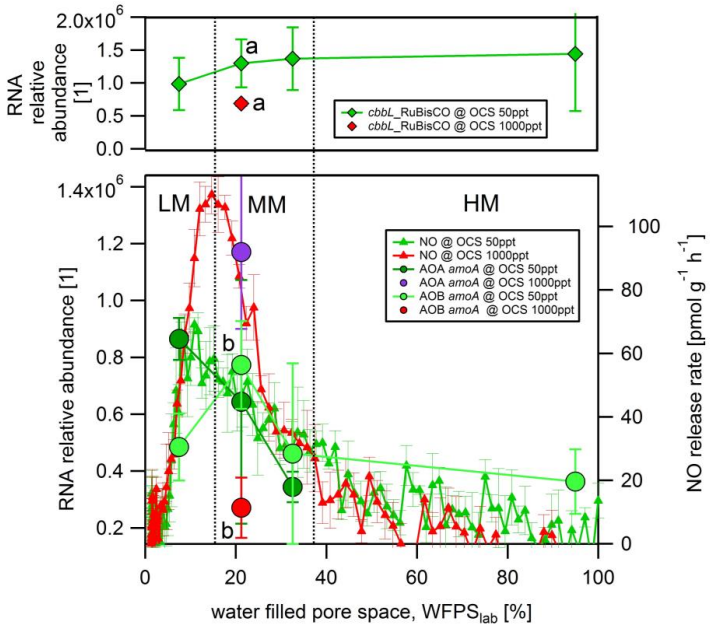

Fig. 5 RNA relative abundance of *cbbL* functional gene, encoding Ribulose-1,5-Bisphosphate-Carboxylase (RuBisCO) large subunit type IA, measured over dry-out under 50 ppt OCS (green diamonds) and 1000 ppt OCS (red diamond). RNA relative abundance of *amoA* functional gene for ammonia oxidizing bacteria (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).

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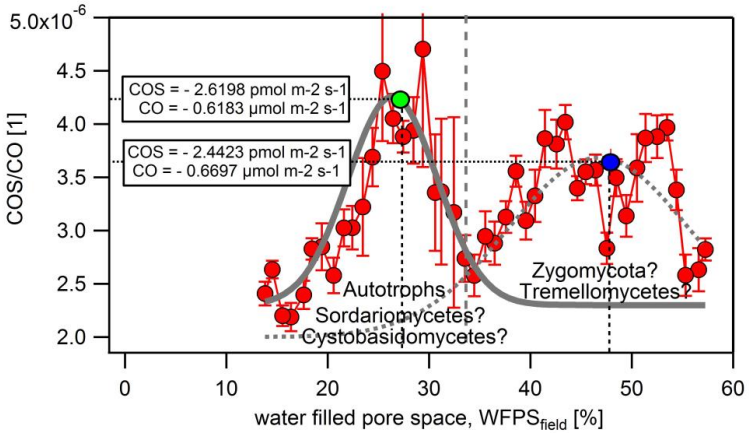

Fig. 6 OCS:CO ratio reanalyzed from chamber measurements from Sun et al. (2017) field data in a Scots pine forest from Hyytiälä normalized by assuming Q_{10}-value equals 2. Just as denitrifiers and nitrifiers affect N_2O:NO ratios differently, we assume autotrophs, Sordariomycetes and Cystobasidomycetes as well as Zygomycota and Tremellomycetes 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).

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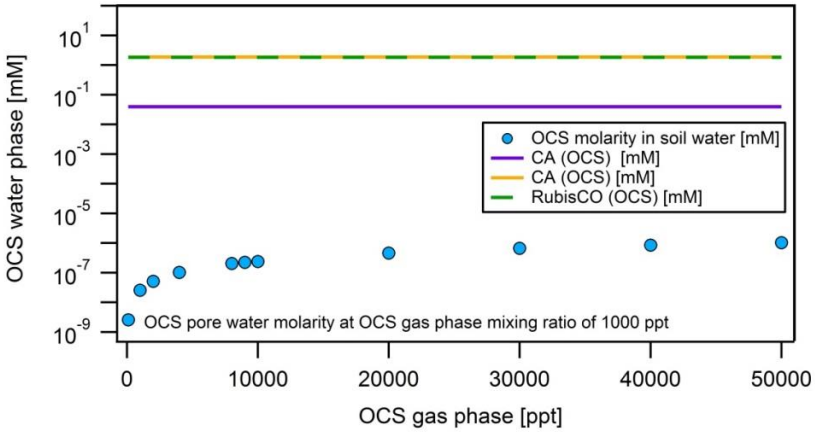

Fig. 7 The range of K_M values of carbonic anhydrase (purple and orange) and RubisCO (green) compared to the calculated OCS concentration in the water phase (blue). The expected water phase concentration was calculated in a similar approach than in Bunk et al. (2017) from the known gas phase concentration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