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Soil carbonyl sulfide exchange in relation to microbial community composition: Insights from a managed grassland soil amendment experiment

Florian Kitz[a,*], María Gómez-Brandón[b,c], Bernhard Eder[b], Mohammad Etemadi[b,d], Felix M. Spielmann[a], Albin Hammerle[a], Heribert Insam[b], Georg Wohlfahrt[a]

[a] Department of Ecology, University of Innsbruck, Sternwartestraße 15, Innsbruck, Austria
[b] Department of Microbiology, University of Innsbruck, Technikerstraße 25, Innsbruck, Austria
[c] Now at: Departamento de Ecología y Biología Animal, Grupo GEA, Universidad de Vigo, Vigo, 36310, Spain
[d] Now at: Department of Horticultural Science, School of Agriculture, Shiraz University, Shiraz, Iran

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ABSTRACT

The viability of carbonyl sulfide (COS) measurements for partitioning ecosystem-scale net carbon dioxide (CO_2) fluxes into photosynthesis and respiration critically depends on our knowledge of non-leaf sinks and sources of COS in ecosystems. We combined soil gas exchange measurements of COS and CO_2 with next-generation sequencing technology (NGS) to investigate the role of soil microbiota for soil COS exchange. We applied different treatments (litter and glucose addition, enzyme inhibition and gamma sterilization) to soil samples from a temperate grassland to manipulate microbial composition and activity. While untreated soil was characterized by consistent COS uptake, other treatments reduced COS uptake and even turned the soil into a net COS source. Removing biotic processes through sterilization led to positive or zero fluxes. We used NGS to link changes in the COS response to alterations in the microbial community composition, with bacterial data having a higher explanatory power for the measured COS fluxes than fungal data. We found that the genera *Arthrobacter* and *Streptomyces* were particularly abundant in samples exhibiting high COS emissions. Our results indicate co-occurring abiotic production and biotic consumption of COS in untreated soil, the latter linked to carbonic anhydrase activity, and a strong dependency of the COS flux on the activity, identity, abundance of and substrate available to microorganisms.

1. Introduction

Anthropogenic CO_2 emissions, which are primarily driving climate warming, are dwarfed by the magnitude of natural carbon cycling (IPCC, 2013). The two major components of the natural carbon cycle are terrestrial photosynthesis, primarily carried out by plants, and respiration by auto- and heterotrophic organisms. The quantification of their contribution to the global CO_2 flux is crucial to estimate future atmospheric CO_2 concentrations, as their balance determines whether land ecosystems act as sources or sinks for CO_2. Traditional approaches to estimate those quantities at scales beyond the leaf, e.g. the ecosystem level, are plagued by several systematic uncertainties (Wohlfahrt and Gu, 2015). A new method has received growing attention during the last decade (Sandoval-Soto et al., 2005; Wohlfahrt et al., 2012; Asaf et al., 2013; Berry et al., 2013; Berkelhammer et al., 2014), which uses the uptake of carbonyl sulfide (COS) as a proxy for the CO_2 uptake by plants and by extension photosynthesis. COS is a trace gas with a mean concentration of 500 ppt in the atmosphere (Montzka et al., 2007) and its diffusion pathway into leaves is similar to CO_2 (Sandoval-Soto et al., 2005; Seibt et al., 2010; Stimler et al., 2010), where both gases react with the enzyme carbonic anhydrase (CA) (Protoschill-Krebs and Kesselmeier, 1992). The advantage of COS over CO_2 measurements is the absence of carbonyl sulfide emissions from leaves of vascular plants, leading to a unidirectional flux which is expected to scale with gross photosynthetic uptake (Seibt et al., 2010).

The enzyme primarily responsible for the consumption of COS in plants is CA (Protoschill-Krebs and Kesselmeier, 1992; Protoschill-Krebs et al., 1996). CAs can be found in plants, vertebrates, bacteria, algae, fungi and archaea (Lindskog, 1997; Smith et al., 1999; Elleuche and Poggeler, 2010; Del Prete et al., 2014; Steiger et al., 2017). CAs catalyze the conversion of CO_2 and H_2O to H_2CO_3 (Tripp et al., 2001). COS, which is not the target substrate of most CA's (see Ogawa et al. (2013) for an exception), is hydrolyzed to H_2S and CO_2 (Protoschill-Krebs et al., 1996). Besides CA, other enzymes are known to hydrolyze COS,

* Corresponding author. Sternwartestraße 15, 6020, Innsbruck, Austria.
E-mail address: florian.kitz@uibk.ac.at (F. Kitz).

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e.g. RuBisCO, nitrogenase, CO dehydrogenase, COSase and CS_2 hydrolase (Lorimer and Pierce, 1989; Ensign, 1995; Seefeldt et al., 1995; Ogawa et al., 2013; Smeulders et al., 2013). The knowledge about enzymatic COS production is scarcer. It is known that the thiocyanate hydrolase mediated degradation of thiocyanate and isothiocyanate can produce COS (Katayama et al., 1992, 1993; Conrad, 1996; Kim and Katayama, 2000; Chaudhari and Kodam, 2010) and the reaction of CS_2 to H_2S and CO_2, catalyzed by an archaeal CS_2 hydrolase, creates COS as an intermediary (Smeulders et al., 2011, 2013).

The use of COS as a proxy for the ecosystem-scale gross CO_2 uptake by plants depends on the assumption that leaf uptake contributes the major fraction of the net ecosystem COS exchange, which encompasses all uptake and emission of COS within the ecosystem. The existence of major non-leaf fluxes would obscure the signal of COS uptake by leaves on the ecosystem level. Soils are potentially significant contributors to ecosystem COS uptake since they harbor high numbers of organisms that produce CA and CA-related enzymes (Ogawa et al., 2016; Meredith et al., 2018b). Kesselmeier et al. (1999) showed COS uptake by soils under different environmental conditions and a reduction of the uptake rate after the addition of a CA inhibitor (6-ethoxy-2-benzothiazole-2-sulfonamide), indicating that soils might act as a sink due to CA activity in soils. Based on those findings, global budgets (Kettle et al., 2002; Montzka et al., 2007) subsequently included oxic soils as carbonyl sulfide sinks. The notion of the soil as a COS sink was further reinforced by empirical data (Sauze et al., 2017; Kaisermann et al., 2018b) and mechanistic soil COS flux models (Ogée et al., 2015; Sun et al., 2015). But the role of oxic soils as a COS sink is not uncontested, as recent work has shown that under dry, hot (Maseyk et al., 2014; Whelan and Rhew, 2015; Whelan et al., 2016; Meredith et al., 2018a; Yang et al., 2018) and high-light (Whelan and Rhew, 2015; Kitz et al., 2017) conditions, oxic soils can become a considerable source of COS. These soil COS fluxes were attributed to a mix of abiotic and biotic processes, with production and consumption co-occurring. Whelan and Rhew (2015) observed increased COS emission after sterilizing their soil samples, indicating abiotic COS production mitigated by biotic COS uptake. This is in agreement with Conrad and Meuser (2000) who suggested that the net COS flux is a result of complex interactions between different processes and cannot be attributed to a single process. Abiotic COS production in oxic soils is usually attributed to thermo- and/or photoproduction (Maseyk et al., 2014; Whelan and Rhew, 2015; Kitz et al., 2017; Meredith et al., 2018a; Whelan et al., 2018) from organic material on or in the soil. The microbial contribution to COS fluxes is still largely unknown, yet advances have been made recently (Sauze et al., 2017; Meredith et al., 2018a, 2018b). COS consumption under ambient COS concentrations has been observed in bacteria, e.g. *Mycobacterium spp.* (Kato et al., 2008), *Dietzia maris*, *Streptomyces ambofaciens* (Ogawa et al., 2016) and fungi (Sauze et al., 2017; Meredith et al., 2018b), e.g. *Scytalidium* sp., *Trichoderma* sp. and *Beauveria* sp. (Masaki et al., 2016). At a COS concentration of 30 ppm, i.e. 60,000 times the ambient concentration, additional bacteria, e.g. *Geodermatophilus obscurus*, *Streptosporangium roseum*, *Streptomyces albidoflavus*, *Thiobacillus thioparus*, *Cupriavidus* sp. (Ogawa et al., 2016), were found to consume COS.

Our knowledge about soil COS fluxes is limited, thus balancing the magnitude of abiotic versus biotic processes is a difficult, but important step to further our understanding of soil COS exchange. The overarching goal of this study is to provide novel insights into the contribution of soil microbiota to the soil COS flux. We aim to relate the abundance of microbial taxa in differently treated soil samples to the measured COS fluxes. To this end we applied different treatments to temperate grassland soil samples to stimulate, inhibit or exterminate the microbiota and measure the resulting COS fluxes. *In situ* soil COS exchange at this same site was investigated previously by Kitz et al. (2017), who reported exceptionally high COS emissions during daytime, which were related to the intensity of the radiation incident on the soil surface. After the gas exchange measurements the bacteria, fungi and archaea present in the samples were identified. The major hypotheses underlying the present work are that (i) the net soil COS exchange is the result of co-occurring gross uptake and release from biotic and abiotic processes and (ii) that treatment-related changes in the abundance and identity of microorganisms correlate with differences in the soil COS exchange.

2. Material and methods

2.1. Soil sampling

Soil samples were taken from a study site (47°7′ N, 11°18' E) located near Neustift (Austria) in the Central Alps at an elevation of 990 m above sea level. The climate is temperate with alpine influences; the average annual temperature is 6.5 °C, the average annual rainfall amounts to 852 mm. The vegetation at the study site is dominated by *Dactylis glomerata*, *Festuca pratensis*, *Phleum pratensis*, *Trisetum flavescens*, *Ranunculus acris*, *Taraxacum officinale*, *Trifoilum repens*, *Trifolium pretense* and *Carum carvi* (Hortnagl and Wohlfahrt, 2014). Across all major plant species and the vegetation period, the interquartile range (IQR) of the plant C/N ratio ranges from 13 to 22, with the group of legumes (*Trifolium pratense* and *repens*) being characterized by a lower C/N ratio (IQR 9–11). The soil was classified as a Fluvisol with an estimated depth of 1 m, the bulk of the roots was located within the first 10 cm. The site, situated on the flat valley bottom, is managed as a hay meadow, with harvests typically occurring at the beginning of June, beginning of August and the end of September. Organic fertilizer is applied in the form of solid and liquid manure during late October each year at amounts corresponding to 2730 kg C ha^{-1} and 341 kg N ha^{-1} (Hortnagl et al., 2018). Three composite samples, consisting out of three cores each, from three plots with similar vegetation, were taken to a depth of 10 cm on the 26-06-2015 (referred to as June samples) and 01-09-2015 (referred to as September samples), sieved (mesh size of 2 mm) and stored at −20 °C (Lauber et al., 2010; Rubin et al., 2013) until the start of the experiment (in February of the following year).

2.2. Physico-chemical analyses

Soil samples were oven-dried (105 °C) for at least 24 h to determine their dry weight (DW). The volatile solids (VS) content was determined by loss on ignition (LOI) in a muffle furnace (Carbolite, CWF 1000) at 550 °C for 5 h. Total C and N contents were analysed in dried samples, using a CN analyzer (TruSpec CHN; LECO, Michigan, U.S.A.). Electrical conductivity (EC) and pH were determined in soil:water extracts (1:5, w/v) by using a conductivity Meter LF 330 WTW (Weilheim, Germany) and a pH Meter Metrohm 744, respectively. Inorganic nitrogen (Ammonium (NH_4^+) and Nitrat (NO_3^-)) was determined in 0.0125 M $CaCl_2$ extracts, as described by Kandeler (1993a, 1993b). Soil characteristics are summarized in Table 1.

Table 1
Initial soil characteristics for the months of June and September. Values are means ± standard deviation.

Soil Parameters	june	september
Water content (%)	32.76 ± 1.94	28.06 ± 4.82
SOM (%)	11.31 ± 1.85	7.60 ± 3.04
pH H2O	6.49 ± 0.02	6.60 ± 0.14
C content (%)	5.89 ± 1.34	3.72 ± 1.57
N content (%)	0.44 ± 0.05	0.30 ± 0.09
Ammonium (µg N/g DW)	3.59 ± 2.41	4.60 ± 1.52
Nitrat (µg N/g DW)	60.47 ± 18.87	27.41 ± 12.69

2.3. Sample treatment

All treatments, litter and glucose addition, CA inhibition and gamma sterilization, were applied to 30 g soil (fresh weight, fw) 2 h prior to the start of the flux measurements, except for the gamma irradiation treatment. In this latter case, the soil samples were gamma sterilized by Mediscan GmbH & Co KG (Seibersdorf, Austria) three weeks before the experiment started and then stored at −20 °C. To prepare the samples for the flux measurements, 30 g of the sterilized soil were extracted under a sterile environment. For the glucose treatment, glucose amounting to 1% of the soil on a dry weight basis was added by carefully spreading it over the soil placed in a plastic beaker and subsequent mixing. The amount of glucose is consistent with Reischke et al. (2015). For the litter + soil treatment, litter, green plant material derived from the same study site (4 month prior to the experiment) and dried at 60 °C, amounting to 3% of the soil on a dry weight basis was added. The amount of litter added was based on the C units compared to the glucose amendment. For the CA-inhibitor treatment 0.15 mg 6-ethoxy-2-benzothiazole-2-sulfonamide (Sigma-Aldrich, Vienna, Austria) was added. The amount and mixing procedure were identical to Kesselmeier et al. (1999).

2.4. Gas exchange measurements

A Quantum Cascade Laser spectrometer (QCL) (Aerodyne Research, Billerica, MA, USA) was used to measure COS and CO_2 concentrations simultaneously. The pressure in the cell was held constant at 20 Torr (≈2666.44 Pa) by a built-in pressure controller. The TDLWintel software (Aerodyne Research, Billerica, MA, USA) was used to operate the QCL, e.g. fitting the absorption spectra, storing and calculating the dry mole fractions and controlling the internal pressure lock of the QCL. The QCL was housed in a temperature-regulated custom-built case (DE Casebuilder.com GmbH, Hamburg, Germany). PFA tubing and stainless steel fittings were used exclusively. The gas measurements were conducted in an air-conditioned lab with temperature set to 22.5 °C. Both June and September samples were split into 3 sets of samples (3 replicates per month) and each set was measured for 8–16 h. Glass tubes were autoclaved (121 °C for 30 min) before the start of each set to avoid cross contamination. Soil samples were filled into a glass tube with a diameter of 28 mm and a length of 150 mm, the glass tubes were wrapped into aluminum foil to prevent light penetration. The glass tubes were connected to a set of valves (SMC Corporation, Tokyo, Japan) controlled by a data logger, programmed to switch the glass tube measured by the QCL every 2 min, on one side and to a humidifier (glass bottles containing acidified distilled water) on the other side. Room air was drawn into the QCL through the humidifier and the sample tube at a mean flow rate of 0.7 sl min^{-1}. While one sample was measured the remaining 6 glass tubes were flushed with the same flow rate. One of the 7 glass tubes was left empty as a blank and measured in between the sample tubes to calculate the fluxes according to Equation (1).

$$F = \frac{q(C_2 - C_1)}{g} \quad (1)$$

F is the soil flux in pmol g^{-1} s^{-1} for COS and μmol $g^{-1}s^{-1}$ for CO_2, q the flow rate (sl/min), C_1 the mean concentration of the empty tube measurements before and after the sample tube measurement (pmol mol^{-1} and μmol mol^{-1}), C_2 the concentration in the sample tube at steady-state (see Suppl. Fig. 1) and g the soil fresh weight in grams. Extensive blank measurements were performed with the system, which revealed contaminations in the setup (Suppl. Fig. 2), likely due to a lubricant used in the valves releasing COS. Based on these data a valve position dependent correction value was developed, which effectively removed this bias (for details see Suppl. Fig. 2) and was added to the concentrations measured in each glass tube during post-processing. The additional uncertainty introduced by this correction, was included in the overall uncertainty of the measurements, which includes instrument uncertainty, flow and weight deviations and uncertainties introduced by averaging.

The system was given 1 h to equilibrate for each set of measurements, data recorded during this period was discarded. The programming language Python (Anaconda distribution, version 4.3.25) was used to calculate the fluxes and the “uncertainties” package to propagate errors.

2.5. DNA extraction

Total DNA was extracted in triplicate from 0.3 g of each soil sample (fw) using the NucleoSpin Soil kit (Macherey-Nagel, Germany) according to the manufacturer's protocol (for each soil sample 0.3 g were stored and from those 0.3 g three replicates were taken). For the γ-irradiated and untreated treatments soil samples were taken before and after the flux measurements, to check if the microbial composition changed during the flux measurements, all other treatments were sampled after the flux measurements. DNA quality was checked in 2% agarose gel and DNA extracts were stored in low-DNA binding tubes (Genuine Axygen Quality 1.7 mL Maximum recovery, Axygen Inc., USA) at −20 °C until use.

2.6. Illumina MiSeq sequencing and bioinformatics pipeline

An amplicon sequencing using Illumina's MiSeq platform was applied to study the taxonomic structure of the three domains (bacteria, archaea and fungi) using three DNA replicates per treatment. The archaeal and bacterial communities were characterized by amplifying a fragment of 16 S rRNA gene capturing the V4−V5 region using the primer set 515 F and 806 R (Caporaso et al., 2011). Fungal communities were analysed by amplification of the internal transcribed spacer 2 (ITS2) region using the ITS3 and ITS4 primer set (White et al., 1990). The cycling conditions were as follows: an initial denaturation at 95 °C for 3 min, followed by 20 cycles (first step) and 15 cycles (second step) of denaturation at 98 °C for 20 s, primer annealing at 56 °C for 30 s, and extension at 72 °C for 30 s with a final elongation step at 72 °C for 5 min. PCR products from all the samples were then purified, quantified and pooled together in equimolar concentrations for sequencing on an Illumina MiSeq instrument using the 2 × 250 bp paired-end approach (Microsynth AG, Switzerland). Raw Illumina MiSeq paired-end reads were assembled with default settings of the PandaSeq software, version 2.88 (Masella et al., 2012). Low-quality reads defined as reads with an average quality score below 25 (for V4−V5 region) and 30 (for ITS2 region), with more than one ambiguous base and a length < 250 and > 260 (for V4−V5 region) and < 285 and > 400 (for ITS2 region) were removed using the Prinseq program, version 0.20.4 (Schmieder and Edwards, 2011). In addition, barcodes and primers were trimmed by the Prinseq software. The processing of filtered reads to operational taxonomic units (OTUs) was done with the LotuS program using USEARCH at 97% similarity (Hildebrand et al., 2014). The taxonomic affiliation of each OTU was obtained by using the SILVA and UNITE Fungal ITS databases for prokaryotic and fungal sequences respectively. Finally, the OTU table was rarefied to the smallest sample size after removing singletons and doubletons (sequences that appeared once and twice respectively). Rarefaction curves can be found in the supplementary information (Suppl. Fig. 3 & 4). For prokaryotic sequences the data of one inhibitor-treated soil was excluded because the number of obtained sequences was not satisfactory.

2.7. Statistics

COS and CO_2 flux data was averaged for every treatment and month and organized in time steps, e.g. time step 1 is the first measurement of the respective treatment. A total of 760 t-tests, using the R package

BSDA and the function tsum. test,were performed to compare the different treatments and months with regard to their COS and CO_2 fluxes to each other taking into account the errors associated with each value and testing at every time step. The alpha value was adjusted accordingly, using the Bonferroni correction, which resulted in an alpha of approx. 6.57×10^{-5}. For each comparison the fraction of time steps with p-values lower than the adjusted alpha is reported. The observed richness, the Shannon index (Shannon, 1948) and the Simpson index (Simpson, 1949) were calculated for the sequencing data. Non-metric multidimensional scaling (NMDS) using Bray-Curtis dissimilarity was used for both prokaryotic and fungal communities. All these analyses were done with the Phyloseq and Shiny-phyloseq packages in R (McMurdie and Holmes, 2013, 2015; R Core Team, 2015).

A machine learning technique called random forest, executed by the R package "randomForest" (Liaw and Wiener, 2002), was used for performing a classification and regression – predicting the mean of measured COS fluxes for each treatment by only taking into account the OTUs, prokaryotic and fungal data were analysed separately, of the respective samples. Afterwards the OTUs were ranked by their importance for this prediction – using the function importance, also included in the "randomForest" package. The data sets were not split and oob values were used for all random forests. A total of 100,000 trees were built with no restriction on the tree depth. In addition significant ($p < 0.01$) fold changes between treatments (pair-wise comparisons, Wald tests) were identified using the DESeq2 (Love et al., 2014) package in R. For this analysis the unrarefied dataset was used, since the package handles library sizes internally (the "normal" shrinkage estimator was used on the data).

3. Results

3.1. Soil gas exchange measurements

On average, untreated soil was a source of CO_2 (0.027 ± 0.006 nmol g^{-1} s^{-1}) and a sink for COS (-0.0008 ± 0.0004 pmol g^{-1} s^{-1}). Compared to the untreated soil samples, the treatments had the following effect: Adding CA inhibitor to the soil increased CO_2 emission (52% of the time steps were significantly different (sig. diff.) in June; 100% sig. diff. in September) and reduced COS uptake (37% sig. diff. in both months) to close to zero (only 26% were sig. diff. from zero in September and 0% were sig. diff. from zero in June). Addition of glucose or litter increased CO_2 (100% sig. diff. in both months and treatments) and COS (95–100% sig. diff. in both months and treatments) emission. Gamma irradiation reduced CO_2 emission (74% sig. diff. in June; 100% sig. diff. in September) and increased COS emission (100% sig. diff. in June; 95% sig. diff. in September), COS emission was close to zero (42% sig. diff. from zero in June, 5% sig. diff. from zero in September). The measured fluxes are summarized in Fig. 1. Of all the treatments only the litter + soil treatment experienced pronounced COS and CO_2 flux changes over the course of the experiment (Fig. 1).

3.2. Next generation sequencing

With regard to *Prokaryota*, the litter added to the soil harbored primarily *Proteobacteria*, *Bacteroidetes* and *Actinobacteria* (Fig. 2, Suppl. Fig. 6). The litter + soil treatments, litter mixed with soil, had higher abundances of *Proteobacteria* compared to all other treatments, in both the June and September samples (Fig. 2). The phylum *Firmicutes* was also more abundant in the litter + soil and gamma treatments (Fig. 2; relative abundance values for each treatment can be found in the supplement, Suppl. Fig. 6). The fungal community, in particular in the litter + soil treatment, was characterized by a high number of unidentified OTUs. The litter + soil treatment had higher abundances of *Zygomycota* compared to all other treatments in both months (Fig. 3). Members of the phylum Ascomycota were the most abundant fungal taxa in all treatments and both months apart from the litter + soil treatments (Suppl. Fig 7).

The NMDS based on prokaryotic abundances separated the June from the September soil samples on the second axis (Fig. 4). On the first axis two distinct groups could be identified, the gamma treated soils and the remaining treatments (Fig. 4). The NMDS of the fungal data did form three groups (gamma treatments, litter + soil treatments and remaining treatments), and did separate the two months (see Suppl. Fig. 8).

The untreated and the gamma treatment samples taken before and after the flux measurements did not show significant differences in richness estimator indices, Shannon index, evenness, and the relative abundance of bacterial and fungal phyla. This is also supported by the fact that these samples did not separate in the NMDS (Fig. 4). Alpha Diversity measures were similar for all of the treatments, except for the litter + soil treatment, which exhibited a substantially lower richness, Shannon- and Simpson indices regarding both bacteria and fungi in the June and September samples (Suppl. Fig. 9). The abundance of fungal OTUs did not change significantly between untreated and litter + soil/glucose treatments. Eleven identified prokaryotic genera had significant positive fold changes in their abundances between the glucose and untreated soil samples (Suppl. Fig. 10), i.e. were more abundant in the glucose treatment compared to the untreated soil. 34 identified prokaryotic genera had significant fold changes in their abundances between the litter + soil and the untreated soil samples (Suppl. Fig. 11), most of them increased in abundance in the litter + soil treatment compared to the untreated soil. For the random forest analyses the gamma treatments were excluded, since they did not contain meaningful information. The random forest classifier identified all treatments, except the inhibitor treatment, correctly, but did not distinguishing between samples of the same treatment taken before and after the measurements (data not shown). Using random forest regression, 42.7% of the variation in the measured COS fluxes could be explained by the bacterial OTUs (both months analysed together). Among the most important predictors were OTUs identified as *Arthrobacter spp.*, Bryobacter sp., *Streptomyces* sp., *Microvirga* sp. and *Cellulomonas sp.* (Suppl. Fig. 12). The relative abundance of these taxa can be seen in Fig. 5. Fungal OTUs explained only 20.0% of the variation, with the most important predictors being unidentified OTUs and OTUs identified as *Mortierella spp.* and *Hygrocybe sp.* (Suppl. Fig. 13). For relative abundances of additional prokaryotic and fungal families, identified by the random forest as important in regard to the COS flux, see the supplementary material (Suppl. Fig. 14 & 15). For the interpretation of all random forest analyses only identified taxa were considered.

4. Discussion

4.1. Biotic and abiotic contributions to the COS flux

One major task regarding COS cycling in soils is balancing abiotic and biotic contributions to the net COS flux (Whelan et al., 2016, 2018). In our study we investigate this question by comparing living soil samples to sterilized ones. The untreated samples in our experiment, which were the treatments closest to natural conditions, acted as a sink for COS across both months and all replicates, which is consistent with previous laboratory studies (Kesselmeier et al., 1999; Whelan and Rhew, 2015) and in accordance with field studies depicting the soil, under similar conditions (e.g. dark, not heated), as a net COS sink (Berkelhammer et al., 2014; Sun et al., 2016). The fluxes were also in the same order of magnitude as reported by Whelan and Rhew (2015). It is however important to note that our soil samples were taken from a fertilized grassland, which puts them closer to soil samples from agricultural systems, which tend to have lower COS uptake rates (Whelan et al., 2016). A recent study by Kaisermann et al. (2018a) showed that nitrogen fertilization can reduce COS uptake and increase COS production.

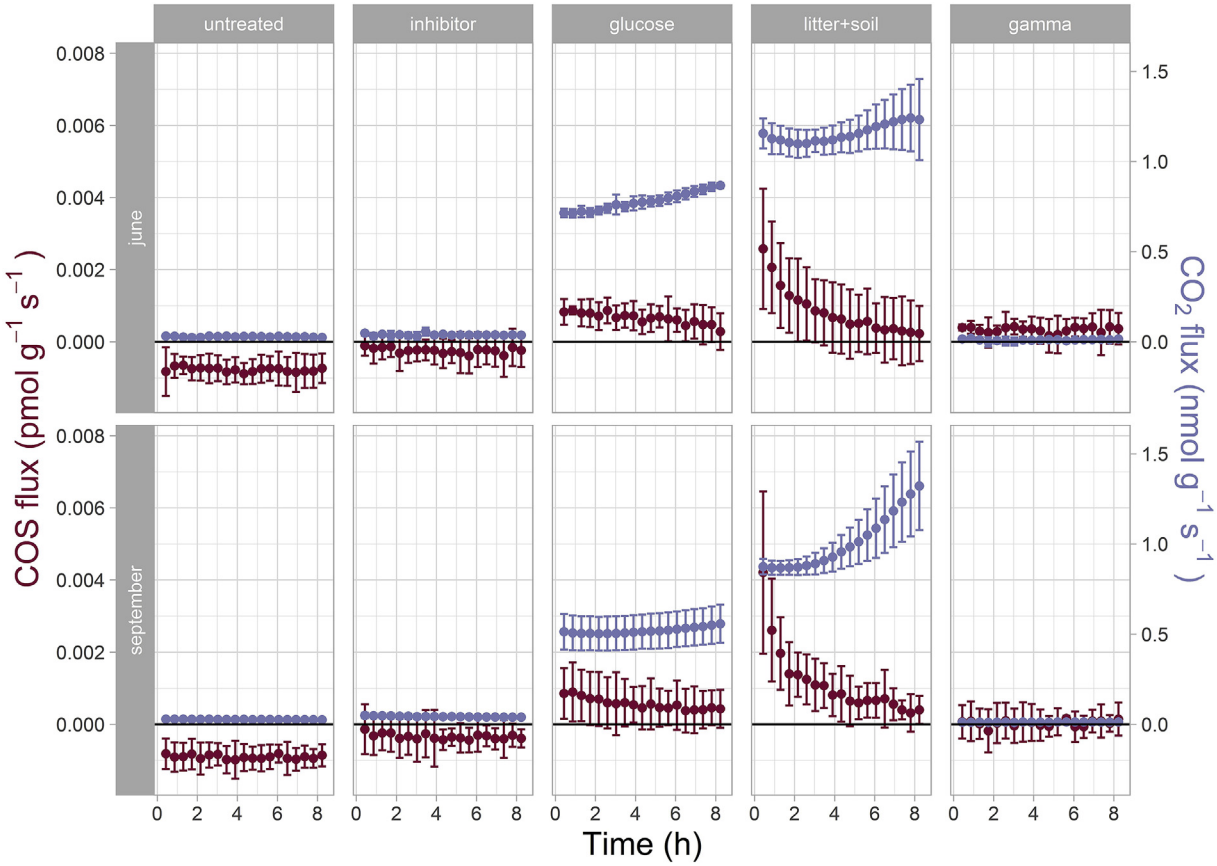

Fig. 1. COS (black, left axis) and CO_2 (blue, right axis) fluxes for each treatment and month. The flux values are means of the three replicates, the errors are a combination of the standard deviation of the three replicates and the propagated errors associated with each flux measurements. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

The gamma-irradiated samples were characterized by a significantly lower CO_2 flux compared to the untreated samples and were close to zero, indicating no or low biological activity. COS fluxes, however, increased in the gamma sterilized samples compared to the untreated samples, even turning the COS flux from negative to positive in some samples (Fig. 1), a similar behavior was observed by Whelan and Rhew (2015). Gamma irradiation was chosen over autoclaving to keep the disturbance of the soil minimal (Trevors, 1996). Since DNA can persist in the soil after sterilization (Levy-Booth et al., 2007; Pietramellara et al., 2009), the genetic data related to the sterilized samples is

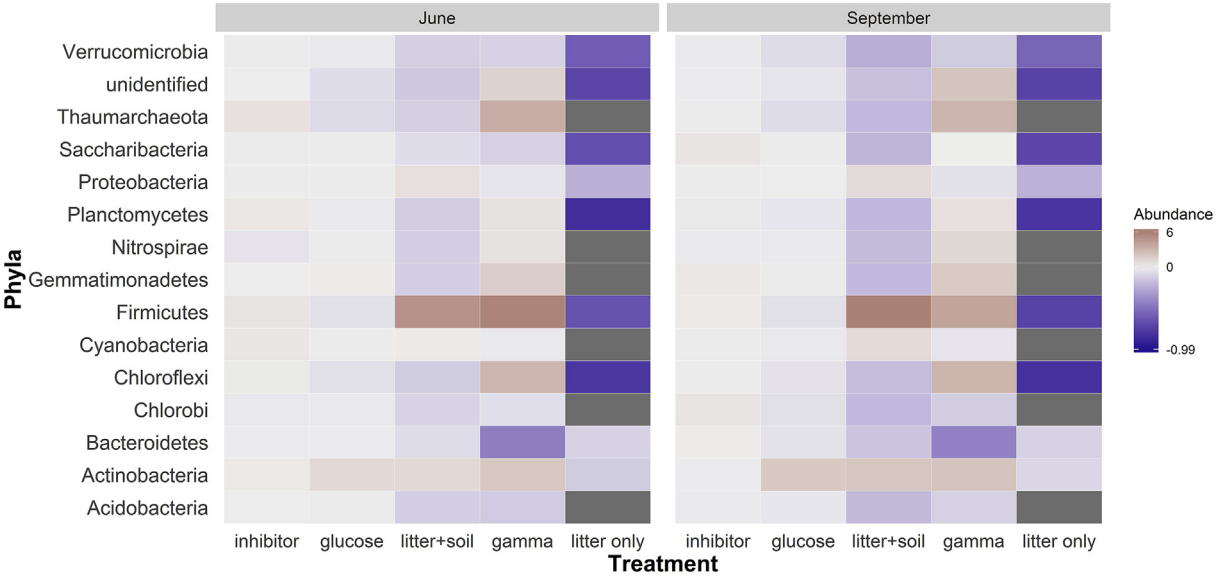

Fig. 2. Heatmap of the differences in relative abundance of prokaryotic phyla between the untreated soil (not shown) and the treated soil samples. Only the top 15 phyla with the highest variability between all treatments are shown. Grey indicates the absence of a phylum from the treatment. (−0.99 corresponds to a reduction of 99% in relative abundance of a specific phylum compared to the control treatment).

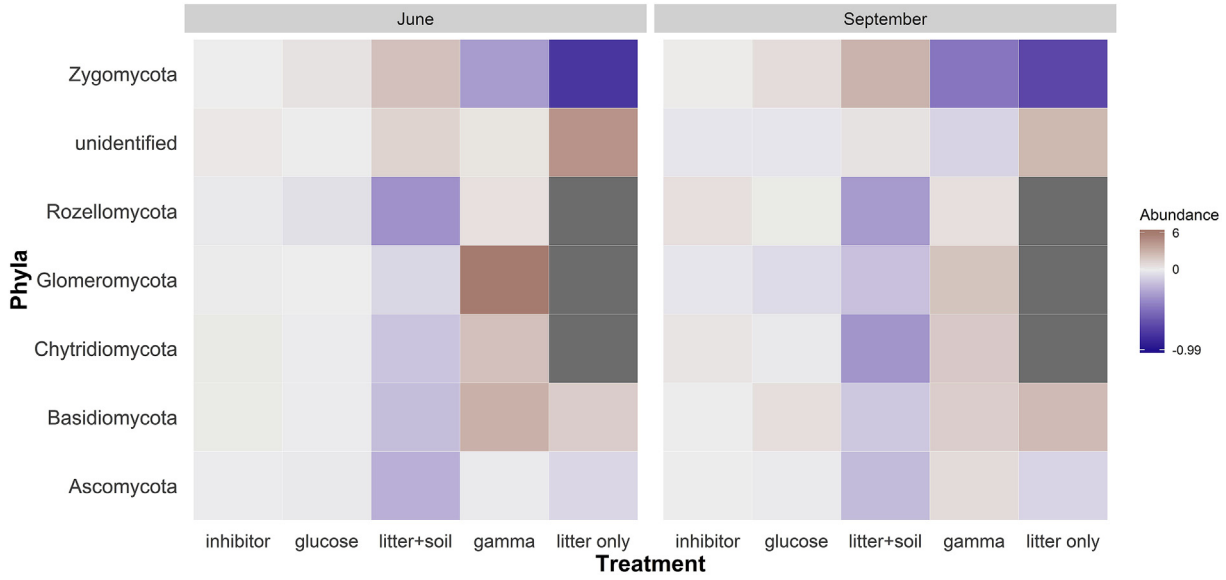

Fig. 3. Heatmap of the differences in relative abundance of fungal phyla between the untreated soil (not shown) and the treated soil samples. Grey indicates the absence of a phylum from the treatment. (−0.99 corresponds to a reduction of 99% in relative abundance of a specific phylum compared to the control treatment).

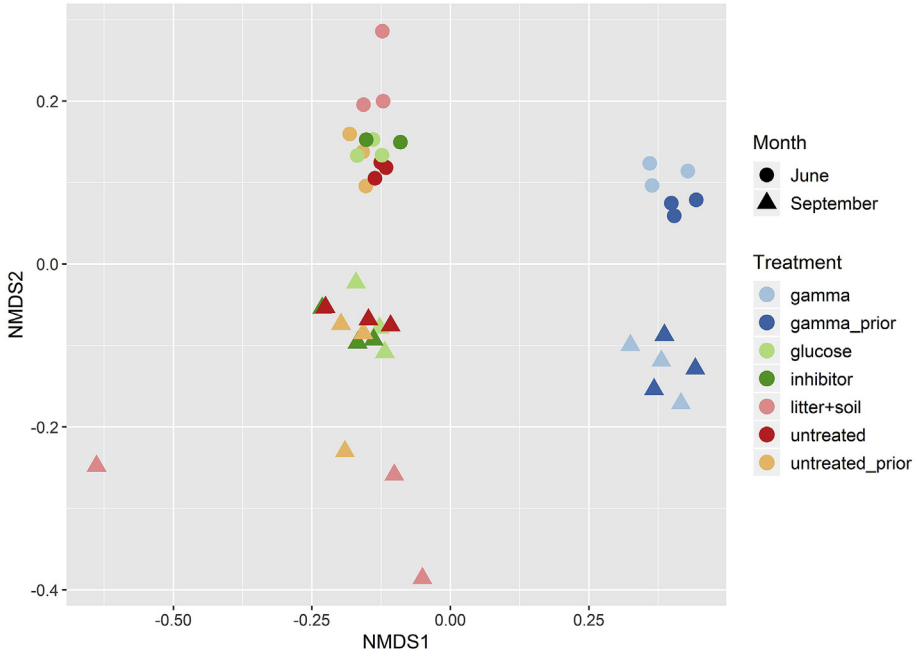

Fig. 4. NMDS, using the Bray-Curtis dissimilarity, of the prokaryotic data with symbols denoting the month and colours denoting the treatment. Treatments followed by "prior" were taken before the flux measurements, all others afterwards (stress value at solution 0.103). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

included only for the sake of completeness. We attribute a large proportion of the observed flux in the sterilized soil samples to abiotic non-photochemical reactions (Jastrow et al., 2007), since substantial biotic production of COS is unlikely in sterilized soil and we can exclude photo-production due to the darkened glass tubes. The observed COS emissions in sterilized samples are in agreement with the proposed COS production mechanism in Meredith et al. (2018a), who suggest that direct COS production is primarily abiotic, while biotic activity produces COS precursor compounds. A small residual biological activity, primarily caused by released enzymes (Lensi et al., 1991; Blankinship et al., 2014) could persist after gamma sterilization, but with the applied dose of 25 kGy most biological activity should have ceased (McNamara et al., 2003). COS fluxes from the sterilized June samples were predominantly positive, while COS fluxes from the September samples were close to zero (Fig. 1). The differences between months, with higher fluxes in the June samples, could be caused by differences in chemical properties (Table 1), amount and quality of substrate and/or a different set of still active enzymes. Especially the higher nitrogen content in the June samples could be responsible for the increased COS flux, since N fertilization has been shown to increase COS fluxes (Kaisermann et al., 2018a).

4.2. CA activity

The majority of COS uptake in soil is most likely related to CA and CA-related enzyme activity (Kesselmeier et al., 1999; Ogawa et al., 2013). Kesselmeier et al. (1999) tested this hypothesis by comparing soils with and without CA-inhibitor addition. We repeated the procedure in our experiment and our results confirm the results of Kesselmeier et al. (1999). The addition of the CA inhibitor reduced COS uptake compared to the untreated soil significantly. CO_2 emission increased in the inhibitor treatment compared to the untreated soil,

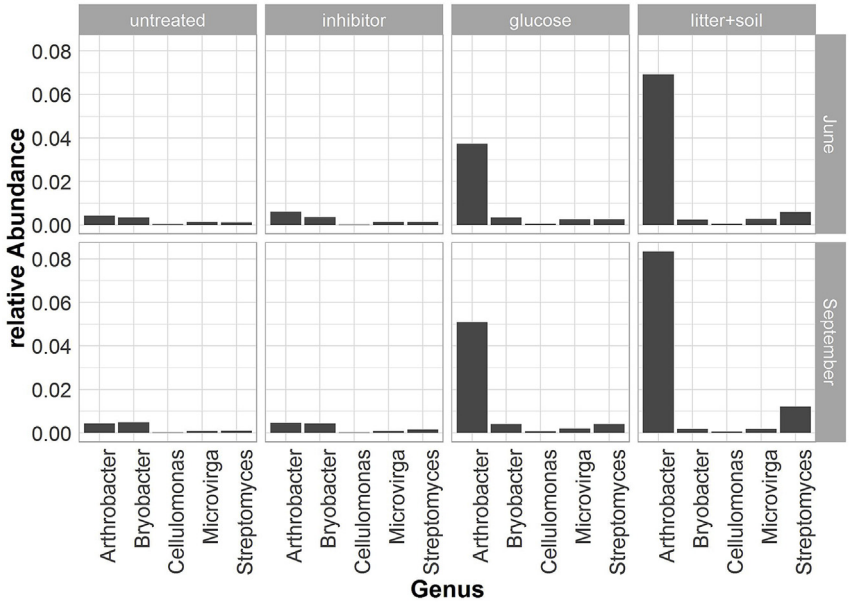

Fig. 5. Relative Abundances of the most important prokaryotic OTUs derived from the random forest analysis.

possibly due to reduced CO_2 uptake by CA. The effect CA inhibition has on microbial growth is largely unknown, considering the reactions catalyzed by CA we expect disruption or slowdown of metabolic reactions (Jones et al., 2017b). If microbial growth is affected by CA inhibition the exposure time in our experiment was too short, since we saw no apparent microbial community compositional changes between the inhibitor and the untreated soil samples. The results from the inhibitor and gamma irradiated treatments in comparison to the untreated soil suggest biological COS uptake at least partially as a result of CA activity.

4.3. Addition of other substrates

Whelan et al. (2016) hypothesized that the accessibility of organic matter in soil has a decisive influence on COS cycling, especially COS production. We provided two additional substrates to the microbial community in the soil samples, glucose and litter. The glucose treatment showed the desired effect of increasing microbial activity, as indicated by the much higher CO_2 fluxes compared to the untreated soil. Surprisingly also the COS emissions increased and became predominantly positive, at first sight conflicting with the proposition of microbial COS uptake as discussed above. Possible explanations are compositional changes in the microbiota, promoting COS producing microbes and/or inhibiting COS consumers, or changes in the microbial metabolism, which in turn could lead to the faster decomposition of sulfur containing compounds. In the treatments with glucose and litter addition, we observed significant changes in the microbial community composition, with Agromyces spp., *Arthrobacter* spp., *Burkholderia spp.*, *Micropriuna spp.*, *Paenibacillus spp.*, *Pseudomonas spp.*, *Rhizobium spp.*, *Rhodococcus spp. and Streptomyces spp*. (Fig. 5) having higher relative abundances in both treatments compared to the untreated soil (Suppl. Fig. 10 & 11). Four of them (*Agromyces spp*., Arthrobacter spp., *Rhizobium spp.*, *Streptomyces spp.*) were also identified as important predictors in the random forest regression (Suppl. Fig. 12). Among them only the genus Streptomyces was previously directly related to COS cycling: Ogawa et al. (2016) demonstrated that a member of the genus *Streptomyces, Streptomyces ambofaciens,* degraded COS under ambient COS concentrations. While Streptomyces spp. would therefore qualify for increased COS consumption, *Arthrobacter spp.* could be involved in biotic COS production in those treatments, since this genus is known to mobilize sulfur (Fox et al., 2016) and produce volatile sulfur compounds (del Castillo-Lozano et al., 2008; Liu et al., 2017), which are known as precursors for COS (Bentley and Chasteen, 2004). Other taxa, which were substantially different in abundance between the treatments, and could be potentially involved in sulfur cycling, like *Pseudomonas spp*. (Ho et al., 2008), were not among the top 20 predictors according to the random forests (Suppl. Fig 12). Literature for the involved species, like *Arthrobacter* and *Pseudomonas* (del Castillo-Lozano et al., 2008; Ho et al., 2008; Liu et al., 2017), indicates primarily indirect COS production through intermediary sulfur compounds rather than direct COS emissions by those organisms, which is in agreement with Meredith et al. (2018a). The flexibility of the microbial metabolism in response to changes in substrate or competitors is well documented (Wang and Levin, 2009; Jones et al., 2017a). Arfi et al. (2003) observed in a yeast cultivation experiment an increase in volatile sulfur compound production after the addition of glucose, as an easily available carbon source. The observation that increased CO_2 fluxes in soil samples, most likely due to increased microbial activity, does not necessarily increase the COS uptake was also made recently in Meredith et al. (2018a). In the litter + soil treatments not only were CO_2 and COS fluxes higher compared to the untreated soil and the glucose treatment, but there was also a pronounced temporal pattern (Fig. 1), which all the other treatments lacked. After an initial delay, CO_2 emissions increased over time. The increase levelled out after ca. 3 h in the June samples, while emissions were still increasing after 8 h in the September samples (Fig. 1). This pattern can be explained by the presence of more complex and recalcitrant compounds in the litter + soil samples compared to the glucose treatment requiring more time and more complex community interactions to degrade (Facelli and Pickett, 1991; Aerts, 1997; Hattenschwiler et al., 2005). On the contrary, COS emissions were very high initially and decreased rapidly thereafter. The initially high COS fluxes could be due to abiotic and/or biotic COS release from decomposing litter, even though the chemical details of COS production in terrestrial systems is not well understood (for aquatic systems see Flock et al. (1997); for terrestrial systems see Meredith et al. (2018a)). The decomposition of litter is likely to produce COS, because sulfur is among the major building blocks of life (Xu et al., 2002), can be found in amino acids, like cysteine and methionine and emissions of other sulfur compounds by soil microbes are documented (Carrion et al., 2015, 2017). The exponential decrease in COS fluxes over time is consistent with a recent field study (Sun et al., 2016) that linked microbial activity, in our study indicated by the CO_2 flux, in

litter to COS uptake. In our experiment the litter + soil treatment did not cause a switch to a COS sink within the 8 h measurement window, indicating that co-occurring COS release still outweighed COS consumption. The decrease in the COS flux appeared slightly earlier than the increase in the CO_2 flux, indicating that enzyme activity responded faster than did microbial growth or that easily degradable sulfur compounds were depleted and therefore COS emission decreased.

4.4. Prokaryotic vs fungal contributions to changes in the COS flux

Microbial community analysis with a random forest regression revealed that prokaryotic OTUs explained 42.9% of the variation in the measured COS fluxes. For the fungal community, the predictive power for the COS fluxes in the random forest analysis was much weaker. But fungi can theoretically play an important role in COS consumption as shown by a recent study (Meredith et al., 2018b). In Meredith et al. (2018b) fungi were found to be more important for CA activity and subsequently COS consumption than bacteria. Our study, in which prokaryotes were much better predictors for changes in the COS flux, might be attributed to lower growth rates of fungi (Rousk and Baath, 2011) and the ability to sustain changes in environmental conditions better than bacteria, reducing the pressure for a community composition change (Barnard et al., 2013; Koyama et al., 2014) during an 8 h time window.

Notable changes in the fungal community composition among treatments included both higher abundances of *Mucoraceae* and *Mortierallaceae* and lower abundances of *Cystofilobasidiaceae* in the litter + soil treatments (Supp. Fig. 15 & 16). The only account, to our knowledge, of one of those families with regard to COS can be found in Masaki et al. (2016), who identified members of the family *Mortierallaceae* as potential COS emitters. The higher abundance of Mortierella spp. in the glucose and litter + soil treatments together with the results of Masaki et al. (2016) adds them to the candidates for biotic COS production. In the random forest analyses for both prokaryotic and fungal data a large number of unidentified OTUs were among the top 20 most important predictors (Supp. Fig. 12 & 13), underlining our lack of knowledge about the soil microbial community in general. Taxa not mentioned here, but associated with COS turnover in previous studies (see introduction), were either present in very small quantities or not present at all in our samples (Suppl. Fig. 17 & 18).

5. Conclusions

The results from the gamma irradiated and untreated soil suggest co-occurring abiotic production and biotic consumption of COS in the investigated soil, supporting the hypothesis that net soil COS exchange is the result of co-occurring uptake and release from biotic and abiotic processes. The biotic COS consumption can partly be attributed to CA activity, because inhibition of CA reduced COS uptake. The addition of other substrates, glucose and litter, turned the soil from being a net sink into a net source for COS, suggesting a decisive role of the quality and quantity of the substrate. In the random forest analyses bacterial abundances explained more of the variation in the observed COS fluxes than fungal ones. Among the bacteria, the genera *Arthrobacter* and *Streptomyces* were consistently more abundant in treatments exhibiting higher COS emissions, suggesting their involvement in COS turnover. The relationship between the measured COS fluxes and the microbial community composition supports our second hypothesis, correlating changes in the COS flux partially to changes in the microbial, especially prokaryotic, community composition.

There are still numerous unknowns with regard to the COS soil exchange, which no study to date was able to fully address, ranging from abiotic chemical reactions to microbial interactions in soil. Further research is for example required for elucidating the processes governing the temporal sequence in gas exchange and the role litter quality and environmental factors (e.g. oxygen availability) play. Future studies should broaden our knowledge by investigating different soils, with their respective distinct microbial communities, investigate the COS turnover capacity of isolated microbial taxa in cultivation experiments and identify the underlying biotic and abiotic chemical reactions.

Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2019.04.005.

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