**The role of soil microorganisms in**

I. Introduction.

II. Disentangling how microbes impact COS fluxes : stakes and methods.

III. COS uptake in soils

IV. COS production in soils.

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Yellow = Kitz et.al

Blue = Kaisermann et.al

Green = Meredith et.al

Red = Behrend et.al

Dark blue = Sauze et.al

**I. Introduction**

Carbonyl sulfide is the most abundant sulfur compound naturally present in the atmosphere, at around 500 parts-per-trillion (ppt). As such, it’s an important component of the global sulfur cycle.

**II. Disentangling how microbes impact COS fluxes : stakes and methods.**

The quantification of their contribution to the global CO2 flux is crucial to estimate future atmospheric CO2 concentrations, as their balance determines whether land ecosystems act as sources of sinks for CO2. Traditionnal approaches to estimate those quantities at scales beyond the leaf (eg: the ecosystem level) are plagued by several systematic uncertainties. A new method has received growing attention over the last decade, which uses the uptake of COS as a proxy for the CO2 uptake by plants and by extension photosynthesis. COS diffusion pathway into leaves is similar to CO2, where both gases react with the same enzyme, **carbonic anhydrase** (CA).

The advantage of COS over CO2 measurements is the absence of COS emissions from leaves of vascular plants (most of them at least), leading to a unidirectionnal fulx which is expected to scale with gross photosynthetic uptake.

As COS uptake by the terrestrial biosphere is dominated by photosynthesis during growing season, fluctuations of atmospheric COS concentrations can serve as a tracer of land photosynthesis.

However the use of COS as a proxy 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.

Soil exchange of COS is temporally and spatially variable, which introduce uncertainty to estimate primary production.

One approach for estimating COS emission rates from soils is to measure the net COS flux rate of air-dried soil samples. This assumes that COS consumption by dry soils is negligeable since hydrolysis by CA require the presence of water to proceed. Thus with a further assumption that COS emissions do not vary while soils are drying. However, it is still not clear whether the COS production by soils is related to biological activty and potentially varying with soil moisture.

In order to measure COS uptake and production, Conrad (1994) proposed an alternative approach that requires the measurement of net COS fluxes at different atmospheric COS concentrations. Since it can hardly be implemented in the field without large artefacts, it is well adapted to measurements on soil microcosms. This method also indicates whether COS fluxes changes with soil water content or not.

**III. COS uptake in soils**

COS uptake in soils relies almost exclusively on biotic processes (since soil sterilization reduce COS uptake), through the activity of microbial enzymes. Carbonic anhydrase is currently considered as the major one since it can be found in a wide range of autotrophic and heterotrophic organisms. As a ubiquitous enzyme for exchanging and equilibrating CO2, CA does not only occur in higher plants but also in microscopic algae and lichens.

CAs are diverse : they include six known classes and participate in carbon fixation, sulfur metabolism and allows homeostatic regulation of intra-cellular pH. These different families have different affinities to CO2 and COS and different expression rates.

CA catalyzes both the reversible hydration of CO2 and the irreversible hydrolysis of COS.

A study from Kitz et.al (2019) on temperate climate soils shows that untreated soils samples were on average sinks for COS.

According to Kaisermann et.al (2018), CA activity rate is mostly related to variations in microbial C biomass since the majority of the smaller COS hydrolysis rates were indeed found in soils with the lowest microbial biomass. The result is consistent with the model of Ogée et.al (2016) that proposes soil CA activity to vary proportionally to the total volume of all the microbes, present in a soil provided that their CA requirements are similar.

Although differences in pH (Ogée et.al, 2016 ; Sauze et.al, 2017) and microbial community structure (Sauze et.al, 2017) may complicate the relationship between the COS uptake rate constant and microbial biomass.

In a recent study from Sauze et.al (2017), COS uptake co-varied with the number of fungal gene copies, a proxy for fungal biomass. There was more copies in alkaline soils, but only in the presence of increased phototrophs.

[copy figure from Sauze et.al]

This could be explain by the fact that fungi could benefit from phototrophs photosynthates, just like ocean bacteria gathering around phytoplankton (phycosphere). Another hypothesis is that the rising of phototrophs algae benefited to potential algivorous fungi that grew in number as a result.

Anyhow, fungi are great at uptaking COS. Meredith et.al (2018) showed that COS catalyzed reaction rate was positively correlated with 19 OTUs from fungal lineages but only 2 OTUs from bacterial lineages. Fungal CAs also seems to catalyze COS hydrolysis faster than CO2 hydration : kcos/kco2 positively correlated with 41 fungal OTUs but only 3 bacterial OTUs).

In contrast, it was negatively correlated with 18 bacterial OTUs and only 4 fungal OTUs.

This supports a role for fungi in OCS consumption, and for algae and bacteria in CO2 exchange. These differences can be attributed to the different classes of CA found in fungal and bacteria lineages. Indeed, trends in COS catalyzed reaction rate were correlated with CA expression levels of B-CA. On the other hand, CA activity for CO2 was instead more related to a-CA than B-CA expression.

That way, it seems that fungi with expression of B-CA are the key players for COS uptake, while bacteria and algae are more effective at exchanging CO2 with CAs from class a.

RUBISCO is also a candidate for COS consumption since it acts as a competitive inhibitor for CO2 uptake by the enzyme. Due to its similarity with CO2, COS can be a direct source of sulfur and/or energy for some autotrophs and heterotrophs.

**IV. COS production in soils.**

COS production in soils starts with the presence of sulfur compounds that act as precursors.

Among them, thiocyanate and thiosulfate seems to play an important role.

According to Lehmann and Conrad (1996), the rising of soil thiocyanate concentration results in an increase of COS production. However, a study from Katayama demonstrated that it can also inhibit COS production. [need to read both of them]

Mechanisms for observed link between sulphur and nitrogen cycling in soils is still not understood. However, it is known that S-containing amino acids such as methionine, cystine and cysteine are all potential precursors for COS.

COS production rates are also related to the physical properties of the soil. Behrendt et.al (2019) showed that COS production rates were higher for soils at water-filled pore space higher than 60%. When WFPS goes down (between 15 to 37%), soils switch from net source to net sink but the relation is not linear since very high WFPS can also lead to a decrease in COS production [need to check].

This is why wetlands are known for being sources of COS, while oxic soils are generally considered to be a sink for COS.

Current studies [which one?] also report that soils can switch between net COS uptake and emission related to soil T°C in addition to soil moisture.

Going back to the study from Kaisermann et.al (2018), results surprisingly shows that all moist soils were net COS sinks at 18°C. But, the COS production rates measured on moist soils were not significatively different than those measured on dry soils.

Instead, COS production correlates with total soil N content, soil redox potentiel and negatively correlated with soil pH.

[insert figure]

The 8 europeans soils with the higher COS production showed high C and N contents, while C and N contents are positively correlated with high microbial C and N biomass and negatively correlated with bulk density.

The exact mechanisms underlying COS production are still under debate but a number of hypothesis have been proposed. This includes the thermal degradation of soil organic matter or desorption of COS from soil surfaces. These propositions are partially supported by the persistence of COS emissions after autoclaving.

Another abiotic process that could lead to COS production is the reaction that occurs in flue gas from molecules present during combustion such as :

CH4 + SO2 → COS + H2O + H2.

Both CH4 and SO2 can be produced in soils, however CH4 is generally produced in anaerobic zones of submerged soils and tends not to accumulate at the soil surface.

It is not clear yet whether this reaction would be possible in dry, aerobic soils.

There is also growing evidence that biotic process could lead to COS emissions. A number of studies provide direct evidence [which one?] for the production of COS during the hydrolysis of thiocyanates when catalyzed by thiocyanate hydrolase, an enzyme found in a range of bacteria.

If COS production rates were even partially driven by such biotic processes, this contribution might be sensitive to soil water content and expected to decrease at very low soil water content as microbial activity tends to slow down and microbes either enter a stationary growth phase and/or a dormant state.

However, no significant reduction in COS production was observed after air drying of the soils.

This might be because some microorganisms can persist for prolonged period of time in drought conditions, utilising reserves at a very slow rate, but remains nonetheless metabolically active.

For exemple, Zoppini and Marxsen (2010) demonstrated that some extracellular activities in river sediments were not reduced after one year of drying. This could be an explanation since air-dried soils can still contain some residual water in soil micropore that maintain enzymatic activity. This could result in a detectable amount of COS emitted.

In addition, Maire (2013) showed that endoenzymes released from dead organisms were stabilized in soils and could still lead to extracellular oxydative metabolism, meaning that even sterilised soils might still produce COS.

**V. Sequencing and further research.**

Metabarcoding, thanks to NGS technologies, let us identify at once all the microbial OTUs present in a soil sample, as long as the different sequences match already known sequences registered in databases.

Three specific markers allows us to focus respectively on bacterial, algal or fungal sequences : 16S, 18S and 23S.

The 23S rDNA plastid marker is especially useful to detect pĥototroph organism sequences since it targets eukaryotic algae AND cyanobacteria.

Sequencing is particularly useful to understand the relation between COS fluxes and bacterial community composition. It allows us to determine, at various taxonomic levels, who’s specifically impacting COS production or uptake rates.

For exemple, fungi are now known for being COS consummers. However, there is some specific fungi that can be strong COS emitters such as Mortierella from the yeast family.

At the same time, some bacteria are suspected to be especially strong COS consummers such as Actinobacteria.

Earlier, we also saw that fungi tends to be more abundant when they’re associated with phototrophs. The next step is now to look at what fungi we can find in these communities, in order to understand how they’re linked to algae and if they’re indeed predatory on them.