Investigating the emissions of soil COS from ecologically and economically important isothiocyanate-producing plant species

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Introduction

Most members of the Brassicaceae family produce glucosinolate, a nitrogen- and sulfur-containing secondary metabolite (Halkier and Gershenzon 2006). They accumulate glucosinolate in their vegetative organs as a form of chemical defence against pathogens and herbivores (Mithen 2001). They are economically important species, particularly over the past 40 years; glucosinolates assume major agricultural significance with the increasing importance of rapeseeds, cultivars of *Brassica napus*, *B. rapa*, and *B. juncea*, as oil crops (Halkier and Gershenzon 2006).

When physically separated compartments containing glucosinolate and a β-thioglucosidase called myrosinase come into contact as is the case when chewing food the enzyme myrosinase hydrolyses glucosinolate which breaks down into the secondary compound, isothiocyanate (ITC, volatile mustard oils) (Barba *et al*. 2016). Root growth accompanied by superficial cell destruction also leads to the release of glucosinolate degradation products (Bressan *et al*. 2009).

These compounds are medically and economically important for humans as they can be used as: cancer-preventing agents (Tang *et al*. 2013); bio-fumigants, to inhibit the activity of unwanted soil organisms (Brown and Morra 1997) and; flavour compounds (Weerawatanakorn *et al*. 2015).

The Brassica, *Alliaria petiolata* (Bieb.) Cavara & Grande commonly known as garlic mustard, is a biennial herb native to western Eurasia. Used as a culinary herb and because of its perceived medicinal value, garlic mustard was probably introduced to North America by early European settlers in the mid-1800s (Rodgers *et al*. 2008). Isothiocyanate has been found in the exudates of garlic mustard roots and it has a negative effect on the seed germination of North American native species (Prati and Bossdorf 2004). These secondary compounds are toxic to some North American mycorrhizal fungi (Callaway *et al*., Wolfe *et al*. 2008) as ITCs induce oxidative stress that can lead to fungal cell death (Calmes *et al*. 2015).

Some microorganisms are involved in degradation of isothiocyanates. Specialised microorganisms are able to rapidly degrade different types of ITC for use as a food source in soil (Warton *et al*. 2003, Matthiessen and Kirkegaard 2006). Recently, Welte and co-workers showed that a phytopathogenic bacteria, *Pectobacterium*, is able to metabolise 2-phenylethyl isothiocyanate (PEITC) as a nitrogen source (Welte *et al*., 2016). In this pathway, when the

enzyme SaxA hydrolyses the PEITC, carbonyl sulfide (COS) is produced. COS is the most abundant sulfur-containing greenhouse gas in the atmosphere, however, because of its low ambient concentration (~0.5 ppb), measurements are difficult compared to other carbon cycle gases (Whelan *et al*., 2018).

Currently mechanisms underlying the release of COS from soils are poorly understood and plant ITC metabolism by soil communities could provide some insights on this process. So far few studies have linked the COS production from soil with ITC metabolism. Thus cultivar soils with plants of the Brassicaceae family could be a terrestrial source of COS influencing the global COS budget if ITC proved to be a precursor of COS.

In this study we measured the COS flux of soils in contact or not with *A. petiolata* an ecologically important plant model of the Brassicaceae family, especially given its current invasiveness in North America (Stinson *et al.*, 2006; Callaway *et al.*, Rodgers *et al*. 2008; Smith and Reynolds, 2014). Differences in COS flux rates were also measured between soils containing *A. petiolata*, soils containing a non-brassica plant species, in this case *Elymus repens* found growing next to patches of *A. petiolata* and bare soils used as control. Furthermore, we decomposed the net COS flux between the rhizosphere and the plant. We addressed the following specific questions:

Is there an effect of plant producing isothiocyanate on net soil COS fluxes?

Is there a difference of COS production and hydrolysis rates between brassica, bare soil and non-brassica plants?

Is there a legacy effect of *A. petiolata* on soil function?

Is the rhizosphere responsible for COS emission?

Is isothiocyanate metabolism a precursor of COS in soils?

Materials and methods

Study species and sampling protocol

Brassicaceae plants are well known to release volatile secondary compounds. Isothiocyanates are biologically active against insects, nematodes, bacterial and fungal pathogens (Blazevic *et al*. 2010). We chose *Alliaria petiolata* (Bieb.) Cavara & Grande as a Brassicaceae model because it has been shown to exude isothiocyanate *via* roots to the surrounding soil environment (Wolfe *et al.*, 2008).

It is fairly simple to find wild populations of *A. petiolata* growing on forest edges and other semi-shaded habitats as well as in the forest understory (Callaway *et al*. 2008). Garlic mustards were collected during the fourth week of February and the fourth week of March. Sampling was carried out around Noaillan in Gironde (33730; -44.486824, -0.358944). During the first sampling campaign at the end of February, five *A. petiolata* patches and their soils were transferred to a set of 5 square pots of 3.5 L. At the same time and locations five further pots were filled with bare patch soils. During the second sampling at the end of March, we transferred four *A. petiolata* plants and their soil to four 3.5L pots and a further four pots were filled with *E. repens* patches and their soil. However, only 3 pots of each sample type were used for gas exchange measurements and statistical analysis. The other pots collected were used to test the gas-exchange measurement protocol and serve as a substitute in case of any unforeseen problems.

To minimize major differences in soil characteristics, samples were collected within an area of approximately 5 x 5 m². To avoid the inhibitory effect of *A. petiolata*, samples of bare soil and *E. repens* were collected at least 30 centimeters away from garlic mustard. This distance was chosen because Wolfe *et al*. (2008) showed that the allelochemicals in the roots of *A. petiolata* did not inhibit mycorrhizal fungi at distances beyond 10 cm of *A. petiolata* patch edges. Samples were kept in a growing chamber at 20°C under 12/12 hours light and dark cycle photoperiod using LED lighting with an intensity of about 500 µmol (photons) m^{-2} s^{-1} in the visible range and sprayed each day with the same amount of distilled water.

Gas-exchange measurements

Assuming that the soil-air COS exchange rate in soil-plant microcosms is the same as in soil microcosms, the same method as described in Kaisermann *et al*. (2018) was used to estimate the net COS flux from all microcosms and the COS production and hydrolysis rates from soil microcosms.

Glass soil microcosms were equipped with screw-tight glass lids fitted with two stainless steel Swagelok® (Swagelok, Solon, OH, USA) connectors fixed to 3.175 mm Teflon™ (Teflon, Chemours, Wilmington, DE, USA) inlet and outlet lines. As set forth in Gimeno *et al.* (2017) microcosms were supplied with dry synthetic air adjusted to the desired COS mixing ratios. The inlet and outlet air streams were analysed sequentially on each microcosm, using a mid-infrared quantum cascade laser spectrometer (QCLS, Aerodyne Research Inc Billerica, MA, USA), and to remove matrix effects caused by water vapour (Kooijmans *et al.* 2016) it was

associated upstream to a Nafion dryer (MD-070-24-S-2, Perma Pure LLC, Lakewood, NJ, USA). A TriScroll 600 pump (Agilent Technologies, Santa Clara, CA, USA) connected to the QCLS with a vacuum line and excess air exhausted to atmosphere was used to maintain flow. The average soil temperature in each microcosm was recorded with a stainless steel temperature probe (3-wire PT100, 15 cm length, 3 mm diameter, reference RS 362 9935). Every 24 minutes, an auto-background was implemented for 120 seconds to prevent instrument drift using a dry N_2 bottle. Using the same N_2 bottle (every 16 to 18 minutes) and an Aculife-treated cylinder (Air Liquide USA, Houston, TX, USA) filled with compressed air and 468.4 pmol (COS) mol^{-1} calibrated to the NOAA-Scripps Institution of Oceanography provisional scale (every 16 to 18 minutes), a two-point calibration scheme was appended. A custom-made multiplexed system (Sauze et al., 2017) was used. Six jars with six different treatments and one empty jar (blank) were measured sequentially over 13h under identical conditions (20°C in the dark) to measure simultaneously 6 different microcosms. The blank jar helped assess whether any of the materials in the system consumed or produced COS during the experiment. Over this period the sequence measured the net COS fluxes from all the microcosms at 3 different COS concentration levels (around 100, 500 and 1000 ppt) to compute the COS production and hydrolysis rates with a linear regression (Kaisermann *et al.* 2018). An acclimation time of 30 minutes following a change in COS was integrated in the sequence, even though only 14 minutes were required to stabilise the COS mixing ratio on the chamber lines after a step change in the COS mixing ratio of the inlet line.

Three inlet/outlet pairs were measured on each microcosm at each COS concentration at an air flow rate φ set at 250 ml min^{-1}. To take into account the residence time of air in the tubing and gas analyser, each line was measured for 120 s and only the last 15 s were kept to compute the mean COS concentration. From each inlet/outlet pair the soil-to-air COS flux was computed as follows: $F = \frac{\varphi}{S}$ (Ca − Cin), where F is the net COS flux (pmol m^{-2} s^{-1}), φ is the flow rate of dry air through the chamber (mol s^{-1}), S (0.00615 m^2) is the soil surface area, C_{in} (pmol mol^{-1}) is the COS mixing ratio on the inlet and c_a (pmol mol^{-1}) is the COS mixing ratio on the outlet. The blank chamber COS flux was never significantly different from zero.

Experiment 1: Effect of *A. petiolata* on the net soil COS flux

Preparation of the microcosms

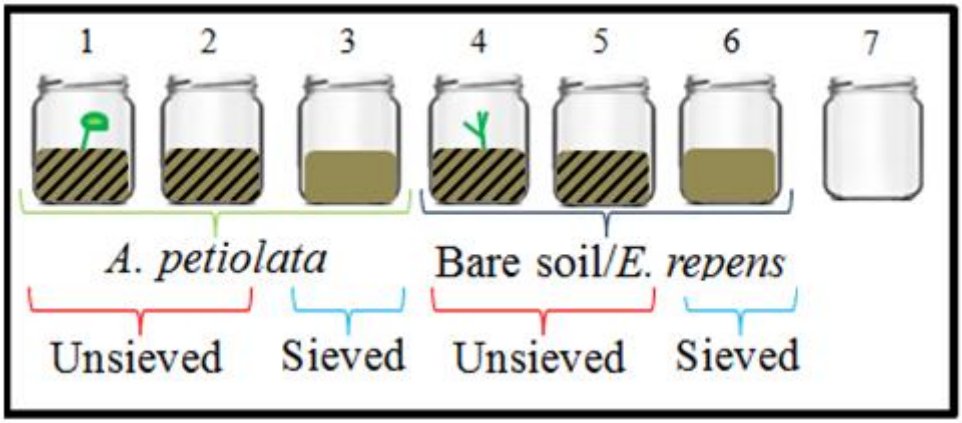
Because of the need for consistent COS flux measurements (Ogée *et al*. 2016; Kaisermann *et al.*, 2018), the same height of soil (5cm) was placed in each of the microcosms. Using the

measured bulk density and respecting the height limit, jars were filled with a similar weight of soil (around 230g of soil). Microcosms were weighed before and after gas exchange measurement to compute the loss in water which was found to be negligible (less than one percent of weight loss).

After each sequence, samples of soil from each microcosm were used to measure its dry weight, pH and redox potential. The remaining soils were then stored at 4°C. After the gas exchange measurement, *A. petiolata* and *E. repens* were also weighed and then used for experiment 3.

Experimental design

There were 2 different types of soil in jars (*A. petiolata* versus bare soil, *A. petiolata* versus *E. repens*) with 3 different treatments: plant with its soil rhizosphere intact, unsieved soil (plant was removed but thin roots were still in the soil) and sieved soil passed through a 4mm sieve (Fig. 1).



<u>Fig. 1.</u> Experimental design: for each sequence, one blank and six microcosms were measured sequentially. Three jars were filled with *Alliaria petiolata* and three others with *Elymus repens* or bare soil. Dashed soils represent those that were not sieved.

Each time, soil in microcosms 1 to 3 came from the same square pot of 3.5L (with *A. petiolata*) and soil in microcosms 4 to 6 were also derived from the same square pot of 3.5L (with bare soil or *E. repens)*. Triplicate measurements of the *A.petiolata*/bare soil couple and *A. petiolata*/*E. repens* couple were carried out (i.e a total of 36 microcosms were measured for COS fluxes). From the first field campaign, gas exchange measurements were only completed on *A. petiolata* and bare soils. From the second field campaign, gas exchange measurements were carried out with *A. petiolata* and *E. repens*.

As ITC are rapidly evaporated or decomposed in soil (Rumberger & Marschner 2003) plants were kept in the microcosm jars. This way the ITC sources were saved. To avoid introducing variability, gas exchange measurements were made in the dark to keep a stable temperature (20°C) and to close the stomata. A preliminary test was carried out to verify that light did not impact gas measurements. With this experimental design, COS flux in soils planted with *A. petiolata* were compared with soils without plant and with a non-Brassica plant comparing the mean net COS flux between the mean of microcosms 1, 2, 3 against microcosms 4, 5, 6. Treatments with sieved and unsieved soils were used to see if there was a legacy effect on soil function.

Effect of *A. petiolata* on soil COS flux, production and hydrolysis rates

Carbonyl sulfide production and hydrolysis rates were calculated by using the method from Kaisermann *et al*. (2018) and the data from the last measurements. This method was adapted to measure COS production and hydrolysis rates in microcosms filled with soil only. In this case, the microcosms with plant and soil were not used. Only the microcosms 2, 3, 5 and 6 were used to calculate COS production and hydrolysis rates.

Experiment 2: Effect of *A. petiolata* roots and its rhizosphere on COS flux

After experiment 1, plants from the microcosms 1 (*A. petiolata*) and 4 (*E. repens*) were removed and placed in new jars. The plant rhizospheres were preserved. The sequence used measured three times at each COS concentration, the net COS flux of one empty jar (blank), one microcosm with *A. petiolata* and its rhizosphere and a last one with *E. repens* and its rhizosphere. After this first measurement, plants were washed to remove rhizosphere and measure the net COS flux of the plant only. The same sequence was applied. To normalize data, the net COS fluxes in this experiment were multiplied by the surface S (0.00615 m^2) of the jar and multiplied by the dry weight of the plant.

Experiment 3: Effect of isothiocyanate on soil COS flux

To test ITC metabolism as a precursor of COS, extra soil collected during the second sampling campaign was sieved and placed in a set of 18 jars for 2 weeks of incubation in a climate-controlled room (MD1400, Snijders, Tillburg, NL) at 20°C in the dark. Jars were weighed and sealed with Parafilm M© to minimise evaporation. During the incubation, jars were opened 3 times a week to add the loss of weight in distilled water and refresh the air in

the microcosm headspace. Previous studies on soils, after the incorporation of Brassica crops as biofumigant, have shown that the potential concentration in ITC is around 100nmol g^{-1} soil (Morra & Kirkegaard 2002). Phenylethyl isothiocyanate (PEITC; _99%) and methanol (>99.9) were purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). This was used to create soil microcosms with a gradient of concentrations in ITCs in the laboratory. With a factor two, the stock solution composed of PEITC, methanol and Milli-Q water was diluted four times. The stock solution was calculated to have a concentration of 100nmol g^{-1} soil after the addition in the jar. A further solution containing Milli-Q water and methanol was kept as a control solution. Because ITC is not soluble in water, ITC was added in methanol where it is totally soluble. This solution was added to the Milli-Q water for the dilutions. Those six solutions were added (5ml) to six jars (a seventh empty jar was kept) and the same sequence as experiment 1 was used to conduct the gas exchange measurements.

Statistical analyses

Data processing and graphs were made with the Rstudio software (R version 3.4.1 2017-06-30) and readr, dplyr, tidyr, ggplot2, RcolorBrewer, gridExtra, plotrix packages. Data with plants that did not follow a normal distribution were analysed using the Kruskall Wallis test and the posthoc Conover test in the PMCMR package. In experiment 2, plants were removed, application conditions were verified and a 2-WAY ANOVA was used and followed by a Tukey HSD test to examine the differences in the net COS flux and the COS production and hydrolysis rates between treatments.

Results

Experiment 1: Effect of *A. petiolata* on soil COS flux

The measured net COS fluxes in Figure 2 represent the uptake and release of COS that occurs in the soil of the microcosms. If the flux was negative, there would be a consumption of COS whilst a positive flux would mean there was a production of COS in the microcosms. Microcosms measured with plant basal rosettes sampled during the first campaign (the winter form of the plant) had significantly different net COS fluxes from microcosms without plants (Kruskal-Wallis test: P=0.003; Fig. 2). In treatments with *A. petiolata* and its soil, the mean net COS flux was positive (357.24 pmol m^{-2} s^{-1}). The treatments containing soil only had negative net COS fluxes (-6.29 pmol m^{-2} s^{-1}).

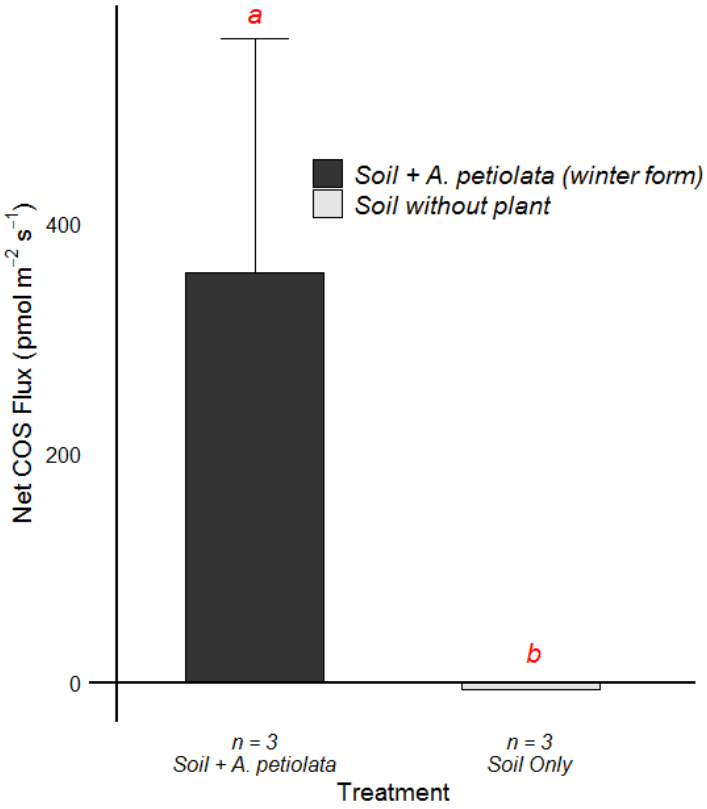


Fig. 2. Variability in microcosm net COS fluxes measured at 20°C and an atmospheric COS concentration of 500 ppt on replicated (n=3) plants and soils sampled from Noaillan, France in February 2018. *A. petiolata* were sampled and measured as basal rosette. Bars represent the mean between triplicate measurements and between each treatment. Bars with different letters are significantly different (P < 0.05) as determined by the Kruskal-Wallis test.

The soil pH was not significantly different between treatments (Student t-test: P = 0.96, Table I). Redox was significantly different (P = 0.01). However, bulk density was almost two times higher in the soil only treatment. Furthermore, the treatment with soil only had a soil dry mass nearly 100g larger than the soils containing *A. petiolata*.

Table I. Soil physico-chemical properties and plant dry mass from the first sampling.

Treatment	Soil dry mass (g)	Plant dry mass (g)	pH	Redox	Bulk density ($g.cm^{-3}$)
Soil + *A. petiolata*	178.246±31.5	1.199±0.9	6.40±0.2	231.3±5.6	0.568±0.1
Soil Only	287.126 ±39.1	0	6.39±0.2	239.7±3.7	0.920±0.1

Microcosms containing soil with *A. petiolata* and *E. repens* from the second sampling also had a significant difference in their net COS flux (Fig. 3). Soils measured in the dark with *A. petiolata* plants had a positive net COS flux (61.24 pmol m^{-2} s^{-1}). On the other hand, soils with *E. repens* plants had a negative COS flux (-15.15 pmol m^{-2} s^{-1}). Net COS fluxes were significantly different between treatments (Kruskal-Wallis test: P=0.0009).

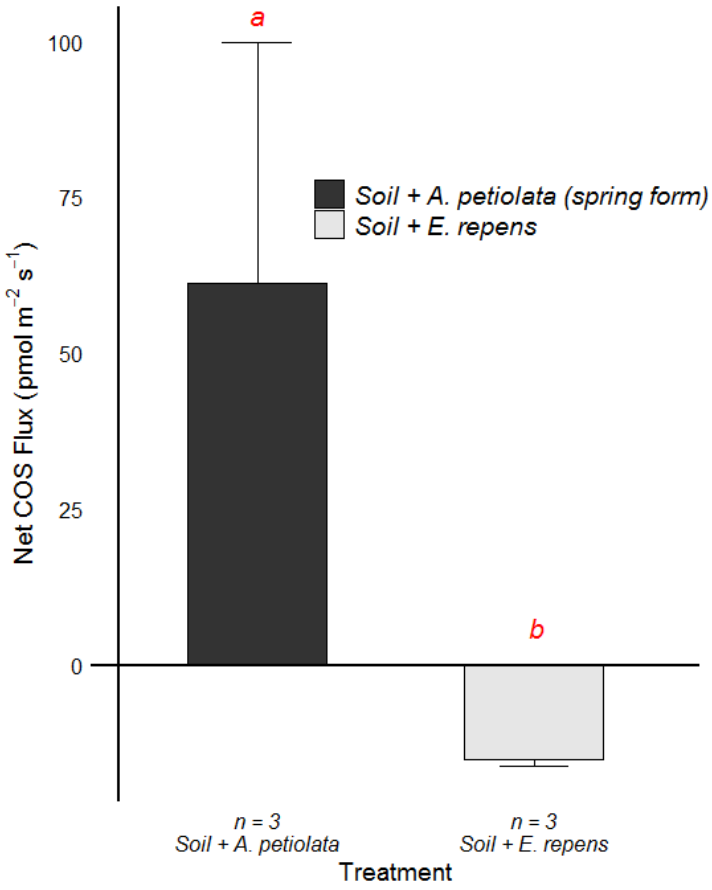


Fig. 3. Variability in microcosm net COS fluxes measured at 20°C and an atmospheric COS concentration of 500 ppt on replicated (n=3) plants and soils sampled from Noaillan in France in March 2018. *A. petiolata* plants during this campaign were measured whilst flowering. Bars represent the mean between triplicate measurements and between each treatment. Bars with different letters are significantly different (P < 0.05) as determined by the Kruskal-Wallis test.

With the exception of redox (Student t-test: P = 0.042) between *A. petiolata* and *E. repens*, the physico-chemical properties between the soils of these two treatments were not significantly different (Table II). Plant dry mass was near two times higher with *A. petiolata.*

Table II. Soil physico-chemical properties and plant dry mass from the second sampling.

Treatment	Soil dry mass (g)	Plant dry mass (g)	pH	Redox	Bulk density
Soil + *A. petiolata*	199.0±17.6	2.079±1.3	7.37±0.1	225.2±13.2	0.647±0.06
Soil + *E. repens*	202.1±9.8	1.122±0.6	7.45±0.2	212.8±10.4	0.657±0.03

There was also a difference in the net COS flux measured between the ontogenetic phase of *A. petiolata* sampled during the two different field campaigns (Fig.4). Treatments with soil and *A. petiolata* basal rosettes (winter form) had a significantly greater positive net flux of COS (Conover test, P = 0.046) than soil and *A. petiolata* in the flowering phase (spring form) (Figs.

2, 3 & 4). Net COS fluxes of bare soil were less negative than the net COS fluxes of *E. repens* and its soils and were also significantly different (Conover test, P = 0.00017).

Fig. 4. ***Alliaria petiolata*** **plants in the basal rosette (left) and flowering phase (right) sampled at the same location in Feb and March 2018, respectively.**

Effect of *A. petiolata* on net COS fluxes and COS production and hydrolysis rates

To test for differences in the COS production and hydrolysis rates of soils only treatments with soil were used i.e. microcosms 2, 3, 5 & 6. Data from microcosms 1 and 4 containing plants were not analysed because, in particular we were interested to explore whether the removal of fine roots from soils and structural disturbance during sieving impacted the net and gross COS fluxes.

First, observing the net COS flux (Fig. 5. a), there was a significant difference between unsieved soils from patches with and without *A. petiolata* (P = 0.021). Unsieved net soil COS fluxes from *A. petiolata* patches were also significantly different from sieved soils from patches without *A. petiolata* (P = 0.032). Unsieved soils, growing on *A. petiolata* patches had a positive flux (0.75 pmol m^{-2} s^{-1}) while sieved and unsieved soils not growing on *A. petiolata* patches always had a negative flux (-3.17 and -3.41 pmol m^{-2} s^{-1} respectively).

Looking at the COS production rate (Fig. 5. b), soils from *A. petiolata* patches had a higher production rate than soils coming from patches without it. COS production rate in sieved and unsieved soils without *A. petiolata* were similar (0.071 pmol kg^{-1} s^{-1}, t.test: P = 0.253) and significantly lower than unsieved soils (0.440 pmol kg^{-1} s^{-1}, P = 0.00001) and sieved soils (0.221 pmol kg^{-1} s^{-1}, P = 0.06) from *A. petiolata* patches. Also soils from *A. petiolata* patche

had a different COS production rate depending on the soil treatment: unsieved soils had a higher COS production than sieved soils (P = 0.004).

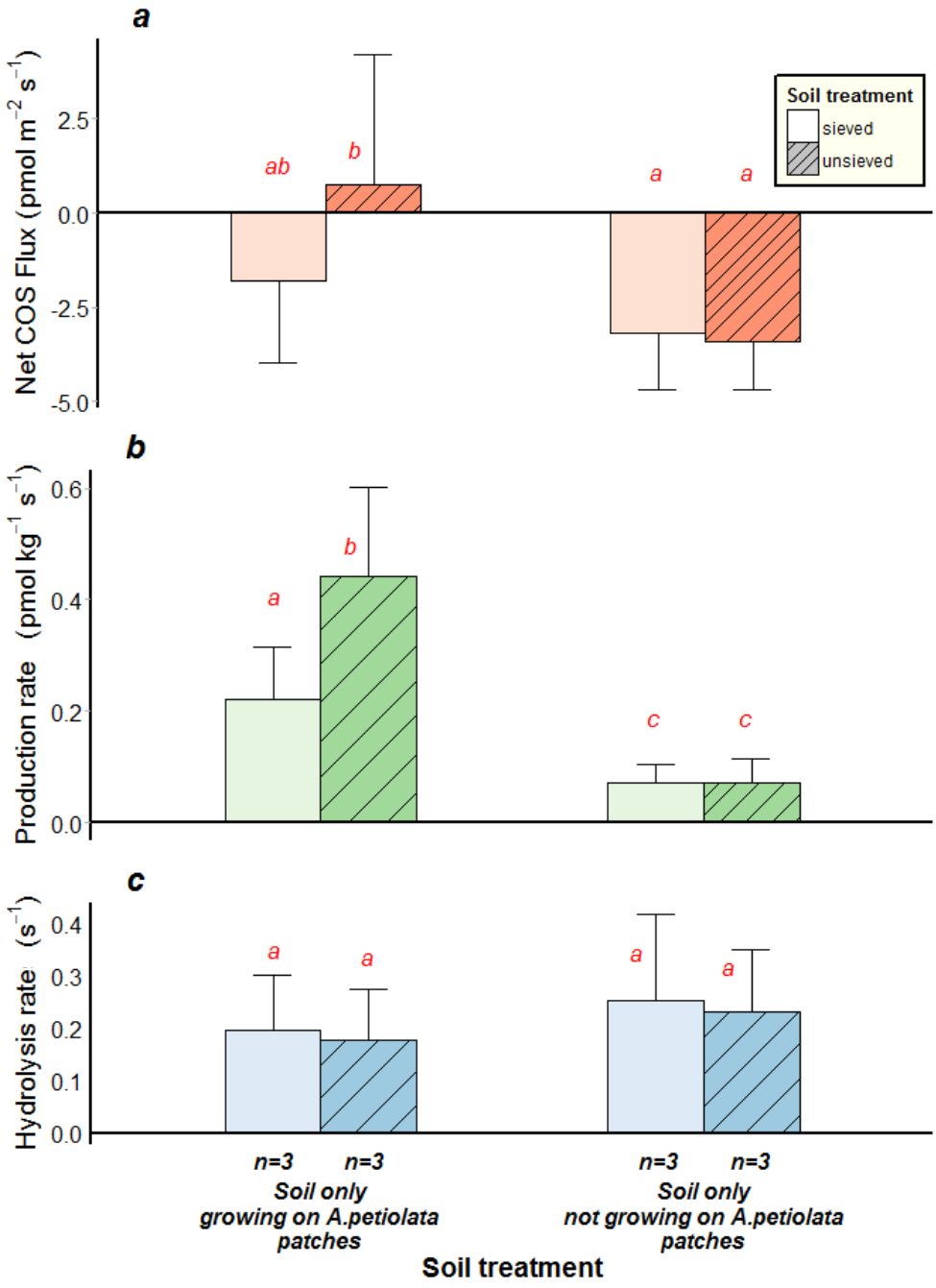


Fig. 5. Variability in net COS flux (a), production (b) and hydrolysis (c) rates measured at 20°C and an atmospheric COS concentration of 500ppt on replicated (n=3) soils sampled from Noaillan in France, February 2018. Bars represent the mean of each treatment. Bars with the same letter are not significantly different (P >0.05) as determined by posthoc Tukey test.

The hydrolysis rate shown in Fig 5. c corresponds to the carbonic anhydrase catalysis of the COS (in s^{-1}). Whatever the soil treatment and where the soil was from, the hydrolysis rate was not significantly different ($P > 0.05$).

Experiment 2: Effect of *A. petiolata* roots and its rhizosphere on COS flux

The net COS flux was measured in microcosms containing *A. petiolata* and *E. repens* with and without rhizosphere (Fig. 6).

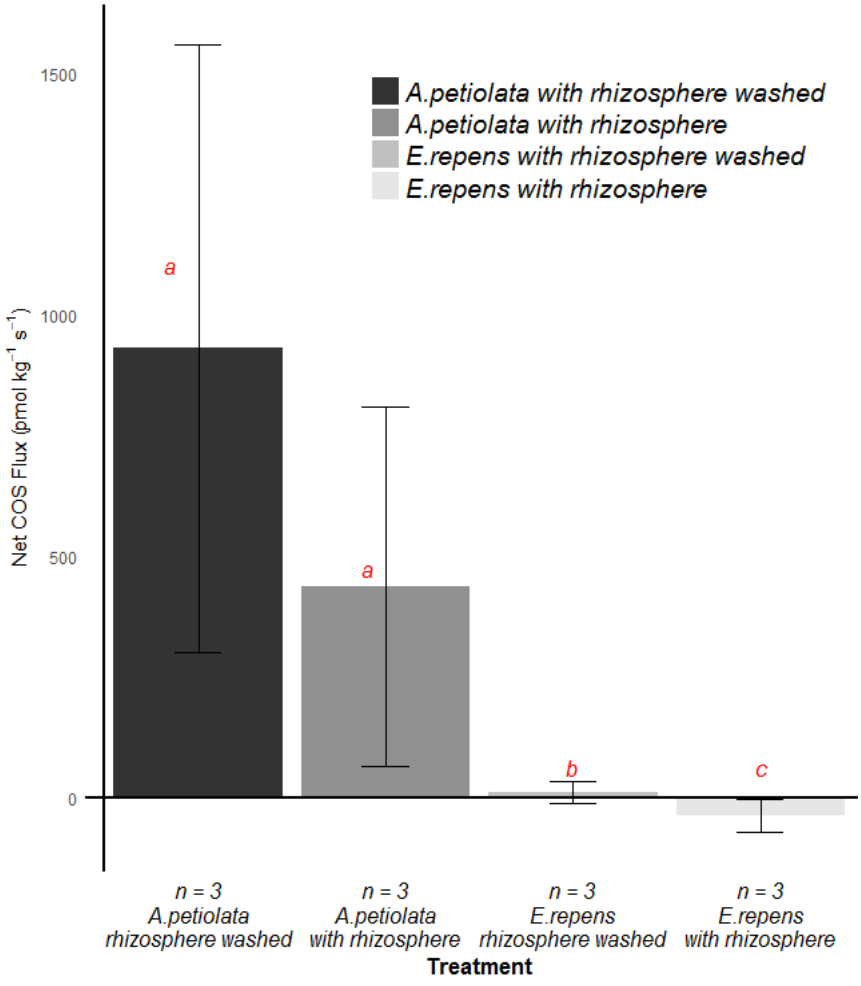


Fig. 6. Variability in net COS flux measured at 20°C in the dark at an atmospheric COS concentration of 500ppt on replicated (n=3) *A. petiolata* and *E. repens* with their rhizosphere washed or not. Bars represent the mean between triplicates of measure and means of each treatment. Bars with different letters are significantly different ($P < 0.05$) as determined by posthoc Conover test.

A. petiolata plants with or without a washed rhizosphere had a positive net COS flux (436.94 and 931.04 pmol kg^{-1} s^{-1} respectively). Irrespective of whether the rhizosphere was washed or not, the net COS flux of *A. petiolata* were not significantly different ($P = 0.203$). It is worth noting that the variability between different plants was large SD here (629.83, 371.88 pmol kg^{-1} s^{-1} respectively).

In contrast *E. repens* plants that were not washed exhibited a negative net COS flux (-37.64

pmol kg^{-1} s^{-1}) that was significantly different to the *A. petiolata* treatments with or without rhizosphere washing ($P < 0.0005$) and also to *E. repens* plants with washed rhizospheres ($P = 0.037$) that exhibited slightly positive fluxes (9.91pmol kg^{-1} s^{-1}). Without rhizosphere washing, the net COS flux of *E. repens* was also different to both garlic mustard treatments (*A. petiolata* with rhizosphere: $P = 0.016$, *A. petiolata* with rhizosphere washed: $P = 0.002$).

Experiment 3: Effect of isothiocyanate on soil COS flux

Unfortunately, results from this experiment were not reliable. The analyser continued to measure a concentration of COS higher than the COS atmosphere concentration even after we stopped the experiment and we removed microcosms to the climatic chamber.

Discussion

The COS budget at the global scale currently needs more observations to improve understanding and modelling of COS sources and sinks. Looking the global budget (Whelan *et al*., 2018) a large source of COS is missing. The oceans could be a large missing source of COS (Sandoval-Soto *et al*., 2005; Berry *et al*., 2013), but there is also evidence that it could be anthropogenic (Campbell *et al.* 2015). Agricultural soils have been shown to be a non-negligible part of the COS emission to the atmosphere (Billesbach *et al.* 2014; Whelan *et al.* 2016).

To the best of our knowledge, soils containing plants producing isothiocyanate like understory forests invaded by *A. petiolata* or agricultural Brassica crops have yet to be shown as soils producing COS.

Our study indicates that soils containing a Brassica plant that has been shown in the past to produce isothiocyanates have emit more COS than bare soils or soils containing other co-existing plant species that are not Brassicas. More specifically, soils containing *A. petiolata* were the only samples that showed greater COS emissions than their uptake of COS. Between soil treatments, soil physico-chemical properties were the same except for the dry mass, redox and bulk density in bare soil treatments. A higher bulk density could reduce the diffusion of COS across soil layers and thus change the concentration of COS in each soil layer because COS uptake by the enzyme CA is dependent on the COS concentration in each soil layer (Ogée *et al*., 2016; Whelan *et al.* 2018). Thus, the negative net COS flux in the bare soil treatment, could be underestimated. In 1995, Devai and DeLaune found that salt marsh soil emitted more COS when redox potential decreased but it was shown in anoxic soils in redox

potential that was negative. Those properties were higher because in the field, only very sandy soils with no living plants were located closely *A. petiolata* patches. *Alliaria petiolata* from the second sampling campaign were in bloom during gas exchange measurements. That could explain why the dry mass of the plant increased while samples between February and March had the same wet weight. We can hypothesise why soils containing *Alliaria petiolata* in the winter form had a net COS flux six times higher than soils with blooming *A. petiolata*. During the winter, *A. petiolata* accumulates glucosinolate, the isothiocyanate precursor in roots to protect itself from herbivores and pathogens. In contrast, when the plant is blooming, it relocates glucosinolates to flowers and leaves to defend its investment in the reproductive system (Smith 2015). Thus, isothiocyanate precursors are hypothesised to be less concentrated in roots during the flowering period reducing the amount of isothiocyanate exuded in soil consistent lower COS emissions in soils containing flowering *A. petiolata* in spring. Only if, in soil, the ITC metabolism is a relevant precursor of COS.

In Figure 5, we compared the net COS flux, production and hydrolysis rates of soil where *A. petiolata* grew or not. Our results clearly show that the COS production is larger than the uptake represented by the hydrolysis rate when the net COS flux is positive. This is also the case for the unsieved soil growing on *A. petiolata* patches. Soils derived from *A. petiolata* patches had a higher production rate than soils without *A. petiolata*. Soils containing *A. petiolata* produced more COS than bare soil or soil growing with a non-Brassica plant. That remains even after we removed the Brassica. The uptake is not influenced by those plants. When we sieved the soil, the production rate decreased if the soil came from *A. petiolata* patches. The process of sieving is to remove, in our case, all the plant living material. This way, thin roots are removed from the soil. If we remove thin roots, we manage to take away the potential source of isothiocyanate to the remaining soil. In this study, with the rhizosphere and roots (a potential isothiocyanate source), there is a strong production of COS however even when the root is removed, the soil function and COS production is still impacted by the past presence of a Brassica plant. Thus there is a legacy effect of *A. petiolata* on soil function. In Figure 5, we can conclude that the net COS flux is dominated by the production rate and not by the hydrolysis rate.

Alliaria petiolata isolated from soils with its rhizosphere washed or not had a net COS flux significantly more positive than *E. repens* under the same environmental conditions. Without soil, *A. petiolata* produced more COS than *E. repens.* This should give us confidence that members of the Brassicas, producing glucosinolates are emitters of COS. Moreover, this emission is not only due to microorganism metabolism as originally hypothesied because we

observed the same production of COS with the rhizosphere washed or not. Interestingly *E. repens* had a greater net COS flux without rhizosphere. Thus we can conclude that the rhizosphere community was responsible for a substantial uptake of carbonyl sulfide. This is consistent with many studies showing that soil microorganisms consume COS. Masaki *et al.* (2016) found a fungi genus from forest soil that exhibited COS uptake. Bacteria in the genera *Thiobacillus, Mycobacterium and Streptomyces* has also been observed degrading COS (Smith and Kelly 1988; Kato et al. 2008; Kusumi, Li and Katayama 2011).The uptake of the rhizosphere could be seen with *A. petiolata* when we look at the mean but, due to the large standard deviation, treatments with *A. petiolata* were not significantly different. With more replicates, maybe we would have seen an uptake of COS by the rhizosphere of *A. petiolata*. In our case, the rhizosphere acted as a consumer of the COS.

It was not possible to say if the isothiocyanate metabolism is a precursor of COS in soil. We thought that synthetic isothiocyanate was put in too high concentration and because of the limit of the analyser; we couldn't say if the really high concentration we saw during the measurement was underestimated or overestimated. It was also possible than the methanol was stuck in the Nafion dryer because of its affinity with water. So, the humidity could go in the analyser. Humidity was able to distort the results. After this experiment, we had to clean the entire measurement device running for 48h atmospheric air in the system. Nafion dryer was also changed.

In conclusion, we have shown that under identical environmental conditions, soils containing *Alliaria petiolata* produced more COS than bare soil and soil where *E. repens* was growing. This effect was still apparent in soil even after the plants were removed. We supposed as others studies (Petersen *et al.* 2001; Rumberger and Marschner 2003; Warton *et al.* 2003; Luang-In and Rossiter 2015) that COS precursors produced by plants for example ITCs still remain in soils even after potential sources such as thin roots are removed. Moreover the soil function was still disturbed. Our work could have been more rigorous if we had used a method enabling us to measure the amount of ITC that was exuded in soil by *A. petiolata*. So, we could confirm that ITC was present in our natural soils. Further studies should now explore the COS production rate and net COS fluxes for other economically important Brassica species such as rapeseed or cabbage. If we cannot refute that Brassicas are a source of COS, agronomic culture with these species could be a significant supply of COS at the regional scale, and bigger than currently estimated (Whelan *et al.* 2017). If so we should not ignore it when modelling the COS global budget. The rhizosphere did not emit COS as net COS flux was increased after we washed the rhizosphere. In future research studies, we will

have to verify if there are no microorganisms after the root has been washed. Microbial communities associated with root system were probably still attached. It had been shown than vigorous washing detached 45% of the rhizoplane population compared to untreated roots (Ritcher-Heitmann *et al.* 2016). If, without rhizosphere and microorganisms, there is still an emission of COS, we will have to consider that ITC could be transformed to COS under abiotic processes. Several studies showed that abiotic processes as high temperatures or light conditions (Whelan *et al*. 2016; Kitz *et al*. 2017) and low redox potential (Devai and DeLaune, 1995), can lead to COS emissions in soil surfaces. Whelan and Rhew (2015) have shown that sterilized soils produced more COS, supporting the abiotic processes leading to a COS production. Moreover, they have demonstrated that some of the COS produced was consumed by in situ microorganisms, supporting our results. Our study was not able to demonstrate if the ITC metabolism in soil is a precursor of COS as we supposed after Welte and coworkers discovery in 2016 of a bacteria enzyme able to metabolise ITC in NH_4^+ releasing COS. Further studies that measure COS gas exchange, should use smaller quantities of ITC realising addition in soil. This concentration has yet to be determined or they can turn to a gas chromatography analysis. Finally, in the case of the North American invasion by *A. petiolata*, on the one hand, if ITC is highlighted as a precursor of COS, this greenhouse gas could be used as a presence sensor of ITC in soil and on the other hand, understory vegetation concerns by the invasion is a large potential source of COS.

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Contributions

When I arrived at the laboratory, analyser, jars for the microcosms, scripts reading and computing gas exchange measurements data that came from the analyser were ready. Sampling protocol, experimentations (preparation of the microcosms, sequences for the analyser, gas-exchange measurements, soil redox and pH measurements), data analysis and report writing have been done by me. The sampling was done with the help of Bastien Frejaville a technician's laboratory and Lisa Wingate my supervisor. Hypothesis and experimentation protocols to test our hypothesis were realised with the help of Aurore Kaisermann my co-supervisor. She helped me to read and interpret raw data that came out of the analyser. Finally, Lisa Wingate assisted me on how to organise my report and present my charts. She reviewed the report and provided advice to improve it.

Abstract

Plants of the Brassicaceae family accumulate glucosinolate and other sulfur-containing compounds in their vegetative organs as a form of chemical defence against pathogens and herbivores. *Alliaria petiolata* (Bieb.) Cavara & Grande (garlic mustard) is a biennial herb of the Brassicaceae family, native to Eurasia and recently introduced to North America in the mid-1800s. Garlic mustard also produces glucosinolate that breaks down into the secondary compound, isothiocyanate (ITC), exuded by roots into the surrounding soil environment and is toxic to North American mycorrhizal fungi. Thus *A. petiolata* is now causing a dramatic change in the composition of North American forests and eliminating tree species that require mycorrhizal partners to grow from the landscape. When ITCs are metabolised by certain bacteria, there is a production of carbonyl sulfide (COS) a greenhouse gas that is present in the atmosphere. Currently mechanisms underlying the release of COS from soils are poorly understood and plant ITC metabolism by soil communities could provide some insights on this process. The aim of this study is to test the hypothesis that soils with Brassicas have higher COS production rates than bare soils or non-Brassicas soils. We compare the rate of COS production from soils containing *A. petiolata, Elymus repens* and a control soil without plants. Soils grown with garlic mustard have a relatively greater COS production rate than soils grown without Brassica plants. This study may help to understand the contribution of plants on the flux of COS between the terrestrial and atmospheric environments.