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Nitrogen Fertilization Reduces the Capacity of Soils to take up Atmospheric Carbonyl Sulphide

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Abstract: Soils are an important COS sink. However they can also act as sources of COS to the atmosphere. Here we demonstrate that variability in the soil COS sink and source strength is strongly linked to available soil inorganic nitrogen (N) content across a diverse range of biomes in Europe. We revealed in controlled laboratory experiments that N fertilisation simultaneously decreases the COS sink strength of soils while increasing the COS production rate. Furthermore, we found strong links between variations in the two gross COS fluxes, microbial biomass and nitrate and ammonium contents, providing new insights into the mechanisms involved. Our findings provide evidence for how soil-atmosphere exchange of COS is likely to vary spatially and temporally, a necessary step for constraining the role of soils and land use in the COS mass budget.

Keywords: COS production; COS uptake; Carbonic anhydrase; nitrate; ammonium; sulfur; microbial community

1. Introduction

Carbonyl sulphide (COS) is the most abundant sulphur gas in the atmosphere [1,2]. Recent interest in the seasonal and spatial variability of atmospheric COS has intensified as its use as an atmospheric tracer of global primary productivity has recently been demonstrated [3–6]. Indeed the enzyme carbonic anhydrase (CA) present in plants catalyses both the hydration of CO_2 and the hydrolysis of COS during leaf gas exchange [3,7–9]. Because CO_2 hydration is the first step of photosynthesis, this COS hydrolysis by plants is expected to be proportional to plant gross primary productivity [5,6,10]. The terrestrial biosphere is estimated to be a strong sink of COS, with around 0.24-1.4 TgS y^{-1} taken up by plants [6,11–13]. Because soil micro-organisms also contain the enzyme CA [14–17], oxic soils have been estimated to take up a further 0.13-0.5 TgS y^{-1} from the atmosphere [6,11–13]. These estimates, however, are based on soil COS flux datasets restricted to just a few studies focused on certain biomes and land use management. To address this problem in spatial representation, new datasets covering a large range of biomes have been collected, leading to clearer spatial patterns of soil COS fluxes at continental scales [18–21]. In particular agricultural soils have been shown to be strong net emitters of COS [19–23] and consequently their behaviour is now described differently from natural soils within global COS budgets [6]. These new recent datasets also helped explain why some oxic soils shifted from being COS sinks to COS sources and how the magnitude of the COS production and uptake varied with soil types [20,21,23], but they also emphasised that the uncertainty on the contribution of soils in atmospheric COS budgets had been largely underestimated.

Thanks to advances made in the partitioning of soil COS uptake and production rates [20,21,23,24], it is now possible to study the drivers of these two opposite fluxes in isolation. It is

known that temperature and moisture are important drivers and can partially explain the observed spatial and temporal variability in soil COS uptake [24,25]. Theoretical considerations hypothesized that CA concentration and the associated gross COS uptake were proportional to the size of the microbial community estimated from measurements of microbial biomass [24]. This hypothesis is supported by recent findings on a range of soils sampled across Europe [21]. In the case of COS production, the drivers are still under hot debate [10] with contrasting evidence supporting either abiotic degradation of COS precursors such as S-containing amino acids [19,20,26,27] and/or biotic [19,21] mechanisms leading to COS production. In the latter case, microbial decomposition of organic sulphur compounds are known to produce COS through different metabolic pathways, including the oxidation of carbon disulphide, thiosulfate [28,29], thiocyanate (SCN^-; [30]) and isothiocyanate [31]. In addition many of these pathways are linked to nitrogen cycling in soils. For example, some of these pathways use nitrate and produce ammonium whilst SCN^- and isothiocyanate are also known to be inhibitors of nitrification [32–34] and CA [35]. Interestingly, a recent finding to emerge from a meta-analysis of European soils indicates that soil N content and mean annual precipitation are the strongest spatial predictors of COS production rates across ecosystems [21], reinforcing the idea that a temperature sensitive coupling between nitrogen and sulphur transformation in soils is driving the magnitude of COS production from soils.

This study set out to test whether the spatial pattern of COS production and consumption observed across land use and biomes was related to the total inorganic N content of soils and whether this result could be replicated in a laboratory manipulation using N fertiliser. Based on our previous findings we hypothesised that, by increasing the inorganic N content of soils, COS production would become an increasingly larger contribution to the net soil COS flux. We also hypothesised that available inorganic N would be positively correlated to the rate of COS production rate and the net COS flux. Finally, because it is also known that NO_3^- can bind to the active site of CA and cause a reduction in CA activity in a range of organisms [36–38], we also hypothesised that N fertilisation would reduce the soil COS hydrolysis rate.

2. Materials and Methods

Soil Microcosm Gas Exchange

To test these hypotheses, we collected the first 10 centimetres of soil from 27 different locations encompassing a range of biomes and land uses within Europe during the summer of 2016 (Table S1). Re-packed soil microcosms for each site (n=3) consisting of 200-300 g of well-mixed sieved dry soil were incubated in 850 ml glass jars under controlled conditions for two weeks at 23°C and 30% of maximum water holding capacity (additional details in Kaisermann et al. [21]).

After two weeks, net COS fluxes were measured at three atmospheric COS concentrations (1111 ± 29 ppt, 557 ± 10 ppt or 124 ± 8 ppt) in order to partition the COS uptake and production following the method described in [21]. Briefly, each microcosm was sealed with a gas-tight glass cap equipped with two stainless steel fittings to connect to inlet and outlet lines of the measurement system. Dry synthetic air was adjusted to the desired COS mixing ratios and the inlet and outlet

airstreams of each microcosm were analysed sequentially using a mid-infrared quantum cascade laser spectrometer (QCLS, Aerodyne Research Inc Billerica, MA, USA). The net COS flux can be estimated as:

$$F = \frac{u}{S}\left(C_a - C_{in}\right),$$

(1)

where u (m³ s⁻¹) is the volumetric flow of air through the microcosm, S (m²) is the soil surface area inside the microcosm and C_{in} and C_a (pmol mol⁻¹) are the COS mixing ratios in the inlet and outlet lines of the microcosm. Because the air space inside the microcosm is assumed to be well-mixed, C_a is also considered as the COS mixing ratio above the soil surface.

On a theoretical point of view, the net COS flux from the soil can also be written as:

$$F = F_0 - V_{d0}C_a,$$

(2)

where F_0 (pmol m⁻² s⁻¹) is the gross flux of COS production, i.e., the flux F when $C_a = 0$, and V_{d0} (m s⁻¹) is the COS deposition velocity onto the soil surface that would occur in the absence of COS production (i.e. if $F_0 = 0$). The gross flux of COS uptake is calculated by subtracting F_0 from the net COS flux F.

From Eq. 2 Kaisermann et al. [21] showed that by performing steady-state COS flux measurements (F) at different COS concentrations (C_a), it is possible to estimate simultaneously V_{d0} and F_0 from the slope and the intercept of a 3-point linear regression between the two variables, leading to the determination of F_0 and V_{d0}. These two variables can further be expressed in terms of a first order COS hydrolysis rate (k, s⁻¹) a COS diffusivity across the soil matrix (D, m² s⁻¹) and a COS production rate (P, pmol m⁻³ s⁻¹) [21,24]:

$$F_0 = \sqrt{\frac{D}{kBq}}\, r_b P \tanh\left(z_{max}\sqrt{\frac{kBq}{D}}\right),$$

(3a)

$$V_{d0} = \sqrt{kBqD}\, \tanh\left(z_{max}\sqrt{\frac{kBq}{D}}\right),$$

(3b)

where ρ_b (kg m⁻³) is soil bulk density, B (m³ m⁻³) denotes the temperature-dependent COS solubility in water [39], θ (m³ m⁻³) is volumetric soil water content and z_{max} (m) is the depth of the soil microcosm. The COS effective diffusivity D is estimated using the empirical formulation of [40] for repacked soils. Then the value of k that satisfies Eq. 3b is obtained using an iterative numerical method (fzero function in the Pracma Package; Brochers, 2017). Finally this k value is introduced into Eq. 3a to estimate P from F_0.

Soil physico-chemical properties analysis

At the end of each gas exchange measurement, the soils were analysed for a range of physico-chemical properties. Microbial biomass for carbon (MBC) and nitrogen (MBN) were estimated as the difference of dissolved C and N contents between fumigated (24h of chloroform fumigation) and non-fumigated soil extracts consisting of 10 g of soil mixed with 40 ml of 0.5 M of K_2SO_4 and shaken for 30 min). We also measured water extractable carbon (DOC) and nitrogen (DN) in these extracts. We assessed dissolved inorganic N (NH_4^+ and NO_3^-) in extracts consisting of 10 g of dry soil mixed with 50 ml of KCl 1M and shaken for 1h. All these measurements were performed using standard procedures at INRA (https://www6.hautsdefrance.inra.fr/las).

Soil nitrogen fertilisation manipulation

A selection of 9 out of the 27 soils measured in the previous experiment were selected to determine the response of soil N addition on COS flux rates (Table S1). For each site a set of three microcosms containing ambient N levels (no N added) and three microcosms fertilised with 5 mg N in the form of NH_4NO_3, (equivalent to adding 125 g N m^{-2} to a 50 cm soil profile as in Ramirez et al. [41]) were prepared and incubated for two weeks at the same conditions as described above. During gas exchange measurement runs, each site and treatment were always completed on paired microcosms and a blank microcosm was always included to check that no artefact from chamber materials occurred.

Statistical analysis

All data processing and graphs were performed using the R software (Version 3.3.3; R core Team, 2015). For all linear mixed effect models, visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality. To avoid pseudo-replication, we averaged the data by site.

We used principal component analysis (PCA) to identify the soil properties that were best correlated to the three COS flux variables (the net COS flux *F*, the COS production rate *P* and the COS hydrolysis rate *k*) across the 27 European soils. Then, we investigated the relative importance of each soil property in explaining these three variables using linear mixed effect models with maximum likelihood estimation (lme function of R package nlme, [42]), taking replicate measurements as random effects and soil properties as fixed effects. Prior to that, all soil data were standardised with *z*-score transformation to avoid excessive influence of magnitude differences between scales. In addition, the normality of each variable was tested using the quantile-quantile plot and Shapiro-Wilk test and the Box-Cox power transformation was used to select the appropriate transformation prior to analysis [43]. First, we evaluated the goodness of models fit of every single soil properties (Table S2). To that end, the explanatory power of each soil properties for the three COS fluxes was measured with the marginal R^2 value and the significance of correlations was tested with an analysis of variance [44]. Then, we used a multimodel inference approach with the MuMIn package [45] to test all possible combinations of predictive models. As we had a total of 81 samples, we included a maximum of 4 predictors in the models. We used the Akaike's Information Criterion corrected for small sample size (AICc) to estimate the likelihood of each model and ranked the models using the 'dredge' command in the MuMIn package. The null models were lme without

predictors. Following Murtaugh [46], two models with a delta AICc less than 2 were considered statistically equivalent. When multiple equally feasible models were found in the candidate models tested, we used model averaging to produce the averaged parameter estimates (average R^2 value), and the relative importance of each predictor was reported. When two predictors were strongly correlated (correlation coefficient greater than 0.8), only the one generating the lowest AICc was retained in the final best model [43,45].

The effect of ammono-nitrate addition was estimated as the percentage difference of the COS fluxes and soil parameters between each treatment and their control.

3. Results

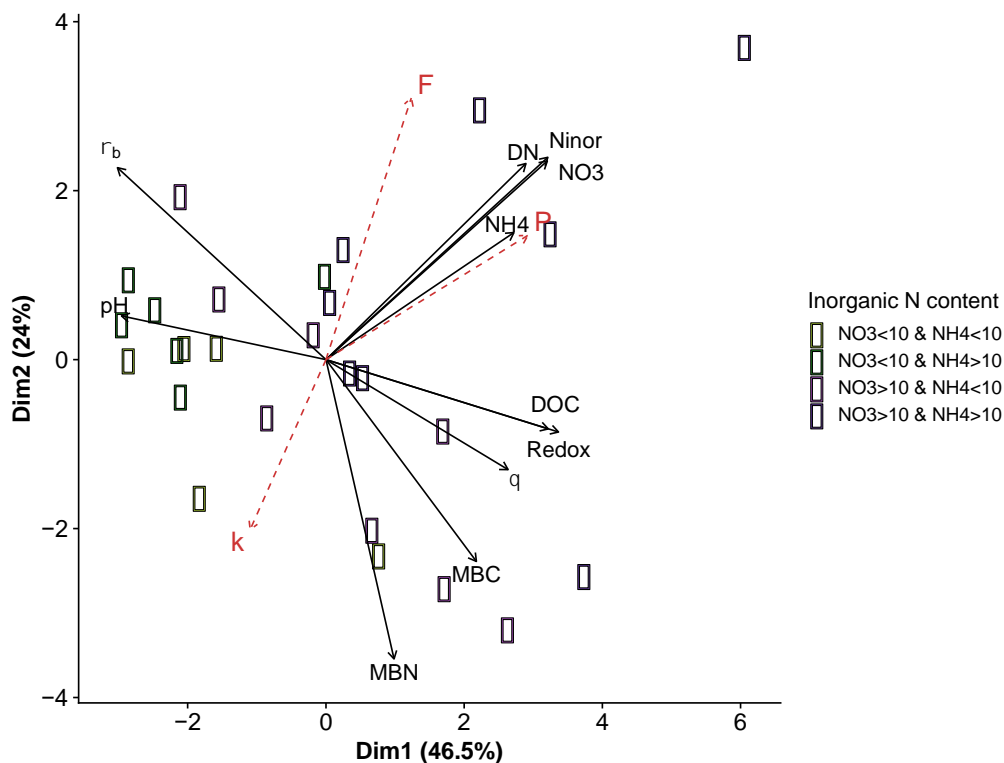*3.1. Variations in soil COS fluxes with inorganic nitrogen across Europe*

Figure 1. Biplot principal component analysis (PCA) of the 27 soils in this study. Each point represents the mean of the three replicates of one soil, colored by level of N-NO_3 and N-NH_4 (above and below 10 µg g^{-1}). Black arrows are the active variables (standardized soil physico-chemical properties) used to build the PCA (ρ_b: bulk density; MBC and MBN: microbial biomass carbon and nitrogen; DOC and DN: dissolved organic carbon and nitrogen; VW: volumetric water content). To investigate the inter-relations between COS fluxes and soil properties, variables of COS fluxes (FCOS: net COS flux F at 23°C; P: gross COS production P at 23°C; k : first-order hydrolysis rate k at 23°C) were fitted as supplementary variables into PCA using the FactoMineR package [47]. The red arrows are the supplementary variables with the coordinates projected onto the PCA and predicted using only the information provided by the performed PCA on active variables.

Our results from 27 sampling sites showed that the net COS flux became less negative when soil inorganic N contents (i.e. NH_4^+ and NO_3^-) increased across European soils (Figures 1 and S1a and Table S2), in support of our first hypothesis. Generally, the weakest net sinks for COS were predominantly found in NO_3^--rich soils also containing high concentrations of NH_4^+ (Figures S1a and S2a). Partitioning of the net COS fluxes into gross COS production (P) and hydrolysis rate (k) indicates a good correlation between P and k with all soil properties related to N (Figure 1, Table S2). Results showed that the increase in soil inorganic N content simultaneously increased the COS production (Figures 1, S1b and S2b) whilst at the same time tended to decrease the capacity of soil to hydrolyse COS (Figures 1, S1c and S2c).

Table 1. Final model statistics explaining the net COS fluxes (*F*), the COS production (*P*) and the COS hydrolysis rate (*k*) across the 27 European soils using linear mixed effect models with maximum likelihood estimation.

Predictors	*F*				*P*				*k*			
	Imp	Coef	*p* value	R²	Imp	Coef	*p* value	R²	Imp	Coef	*p* value	R²
MBC	1	0.61	**<0.0001**	0.69	0.18	0.05	0.49	0.66	1	-0.57	**<0.0001**	0.64
NO3	1	-0.63	**<0.0001**		1	0.47	**<0.0001**		1	0.54	**<0.0001**	
NH4	0.55	-0.13	0.1		0.15	0.14	0.18					
pH	0.13	-0.1	0.2		1	0.39	**<0.0001**		1	0.31	**<0.0001**	
MBC : NO3	0.48	0.15	0.06						0.58	-0.21	**0.02**	
MBC : NH4	0.13	0.13	0.17									
NO3 : pH	0.13	-0.22	**0.04**		1	0.29	**<0.0001**		0.3	0.23	**0.049**	

MBC: microbial biomass carbon. Imp: relative importance of the predictor variables calculated as a sum of the Akaike weights over all of the best models in which the parameter of interest appears (model.avg function in MuMIn package), a value of Imp=1 means that the predictor is present in all best models (AICc <2); Coef is the selected-model averaged parameter estimates (model.avg function in MuMIn package), positive value shows a positive relationship; *p* value for each parameter is adjusted for all other parameters in the selected conditional average model (model.avg function in MuMIn package). R² is the average R-squared value of the selected model (r.squaredLR function in MuMIn package).

The best model explaining the COS production across European soils included the NO3⁻ content, pH and their interaction (Table 1). In particular we observed an increase in COS production with an increase in NO3⁻ content, but modulated by pH as alkaline soils always had low COS production (Figure 2a). However alkaline soils are highly correlated with very low NH4⁺ content, consequently 4 of the 6 alkaline soils were very limited by NH4⁺ content , and therefore we can not exclude an NH4⁺ content effect at high nitrate content for COS production. The best model explaining the COS hydrolysis rate equally included microbial biomass C (and its co-linear microbial biomass N), NO3⁻ content and pH (Table 1). Results showed that the COS hydrolysis rate constant *k* increased linearly with microbial biomass C but with different slopes depending on soil NO3⁻ contents and pH (Figure 2b). The hydrolysis rates were larger on soils with low NO3⁻ content than high NO3⁻ content while for soils with high NO3⁻ content, the hydrolysis rates were larger on acidic soils than alkaline soils.
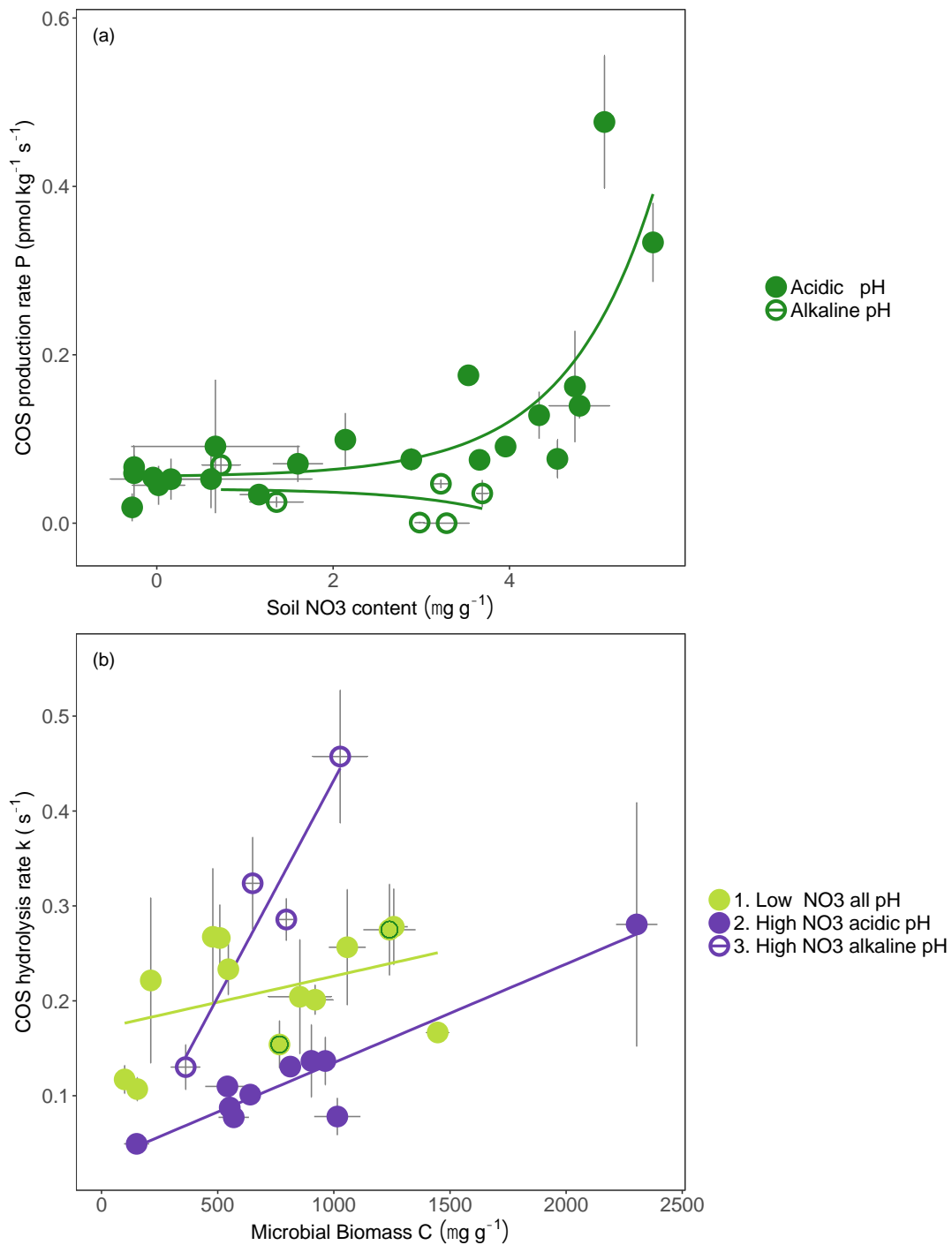
Figure 2. Plots of the best model explaining (**a**) the COS production (P) and (**b**) the COS hydrolysis rate constant (k) measured at 23°C on 27 European soils. Each point represents the mean ± standard deviation for each soil (n=3). Darkgreen circles represent the two alkaline soils with low nitrate content. MBC represents the microbial biomass C and NO3 is the soil N-NO$_3^-$ content (in log). The grey areas indicate the 85% confidence intervals for the exponential (a) and linear (b) models. Low (respectively High) NO$_3^-$ contents correspond to concentrations below (respectively above) 10 µg g^{-1} of N-NO$_3^-$. Acidic (respectively alkaline) pH correspond to pH below (respectively above) 7.

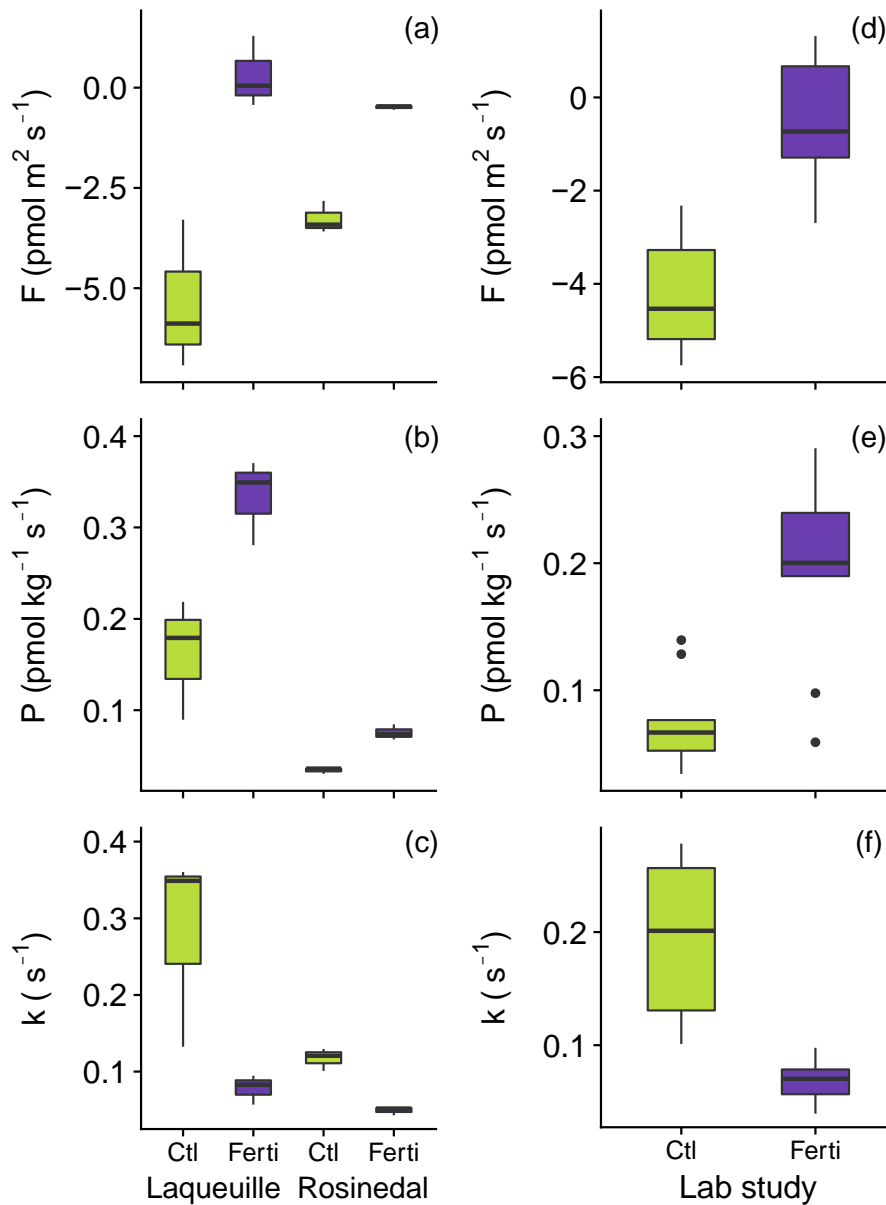*3.2. Impact of N fertilisation on partitioned soil COS fluxes*

Figure 3. Effect of N fertilization (Anova *p* < 0.05) at two field sites (Laqueuille and Rosinedal, n = 3) and in the lab study across 9 soils (addition of ammono-nitrate, n = 27) in the (**a, d**) net COS flux (*F*); (**b, e**) the COS production rate (*P*) and (**c, f**) COS hydrolysis rate (*k*).

A few soils collected from sites with long-term field manipulations of N inputs (Rosinedal a boreal forest soil and Laqueuille a grazed grassland) consistently showed higher COS production rates and lower COS consumption rates in the field-fertilised plots (Figures 3a,b,c). The interaction between soil N inorganic content and COS flux rates were further tested in the laboratory by incubating a subset of nine European soils with ammono-nitrate addition (corresponding to an extra 125 gN m^{-2}). This experiment clearly indicated that the addition of N fertiliser on soils systematically decreased the net COS sink strength of soils (Figure 3d), increased the production of COS (Figure 3e) and decreased the capacity of soils to take up COS (Figure 3f).
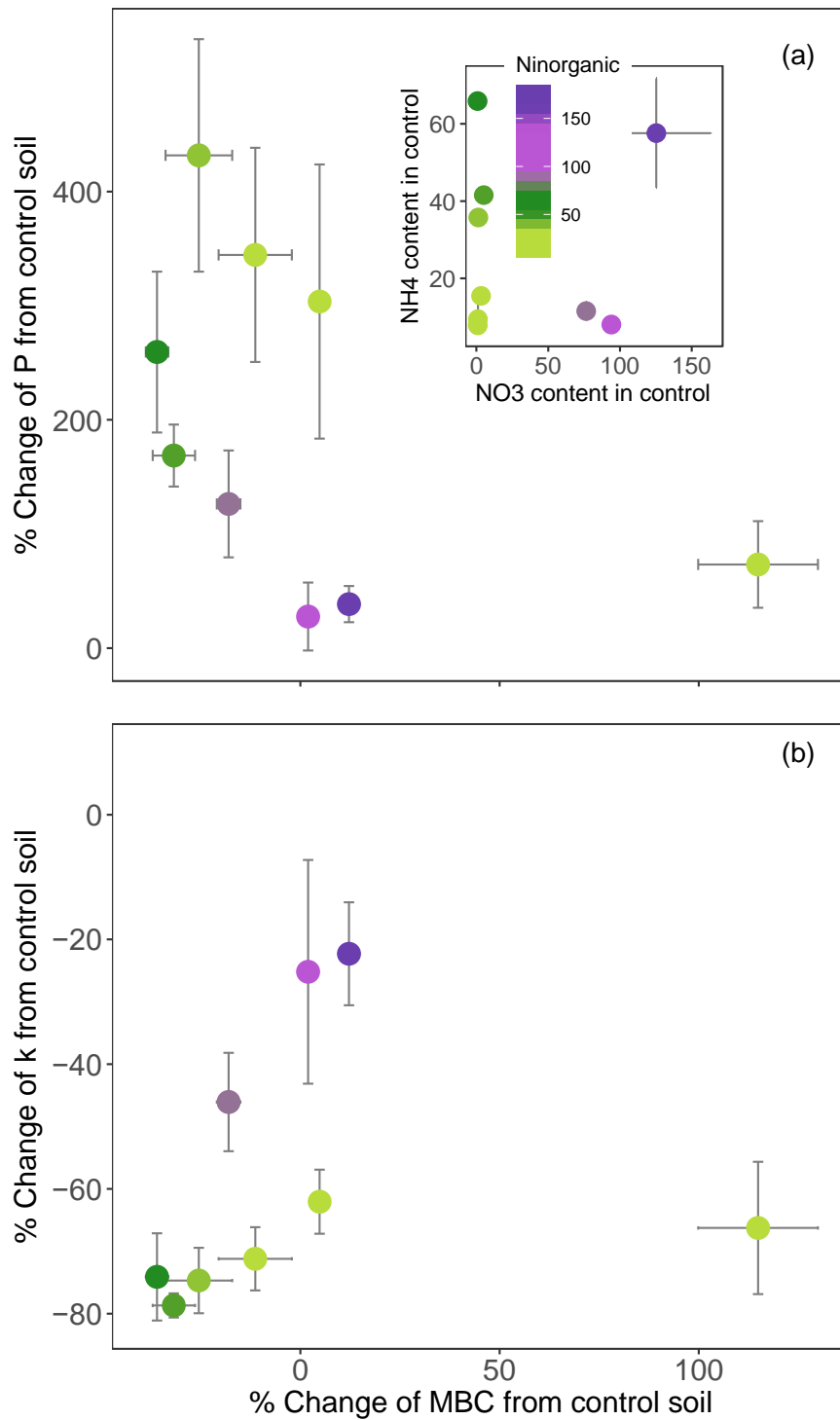
Figure 4. Relationships between the percentage change in (**a**) COS production P and (**b**) COS hydrolysis rate k and the percentage change in microbial biomass C following ammono nitrate addition. Each point represents the mean ± standard deviation for each soil type (n = 3). The colour represents the total N inorganic content ($N_{inorganic}$, in µg g^{-1}) before N addition (i.e. in the control soils), that is the sum of the nitrate (NO$_3^-$) and ammonium (NH$_4^+$) contents (see inset subplot in a).

For both COS production and hydrolysis rate, the relative changes with the N addition depended on the initial inorganic N content of the soil prior to fertilisation and the loss of microbial biomass C (Figure 4a,b). The effects on COS fluxes were stronger when the decrease in biomass was

strongest (Figure 4a,b). In addition, the relative change in the COS production and hydrolysis rate was:

1. larger for soils poor in NO_3^- relative to those rich in NO_3^- (for the same level of NH_4^+);
2. larger for soils poor in NH_4^+ relative to those rich in NH_4^+ (for the same level of NO_3^-) and;
3. limited in soil initially rich in both NH_4^+ and NO_3^- (Figure 4a,b).

4. Discussion

Overall, for the three different scales in our study (field sampling, field fertilisation and lab fertilisation) we demonstrated that high total inorganic N levels consistently reduced the net COS sink capacity of soils. This is also consistent with the study of Melillo and Steudler [26] who reported a significant shift in net COS fluxes after nitrate additions, resulting in COS emissions to the atmosphere in two forest soils within Harvard forest. In contrast, a study by Simmons et al. [48] on boreal forest soils showed that N fertilisation could also result in an increase of the soil net COS uptake rate. However, because gas exchange measurements were performed nearly 16 months after the N fertilisation, it is not entirely clear whether the legacy effect of N additions would be apparent after such a long period as substantial turnover and losses of N fertilisers may have occurred over this period, through leaching and reduction of nitrate.

Mechanisms inhibiting the COS hydrolysis rate

Currently the gross uptake of COS in soil is hypothesised to be driven by the enzyme family of carbonic anhydrases and other homologous enzymes (e.g. COS hydrolase [49]) and CS_2 hydrolase [29]. This hypothesis has recently attained some gravity from a novel metatranscriptome analysis on seven soils outside Europe measured under similar climatic conditions to those made in this study [23]. Meredith et al. (2018b) showed that the putative CA activity of COS was correlated with the presence of β-CA genes (clade D), the dominant class of CA found in all the sampled soils. They also found that CA activity was strongly correlated with soil total N content and C:N ratio, consistent with this study and others [21,50]. In this context laboratory experiments have previously revealed that the activity of CA in a range of different organisms (stripped *Commelina communis* leaves [37], humans [36], fungi [51], methanoarchaeon [52] and insects such as mosquitos [38]) becomes inhibited as NO_3^- concentration increases. This is because anions such as nitrate can bind to the active site of CA and thus inhibit the rate of CO_2 hydration [53]. Our results from the field sampled soils support the hypothesis that the NO_3^- applied in the form of ammono-nitrate may have inhibited COS hydrolysis in a similar manner to that of CO_2 in the above studies.

Although the inhibition of COS hydrolysis by nitrate content in the 27 field-sampled soils was significant, a major factor influencing the COS hydrolysis rate was also microbial biomass C (and its co-linear microbial biomass N). Soils with high microbial biomass content exhibited higher CA activities (Figure 2b). This was further supported with the results of the lab fertilisation experiment that showed that, when the COS hydrolysis rate was systematically reduced by N fertilisation (Figure 3f), especially in soils initially poor in nitrates (Figure 4b), the soils also exhibited a decrease in microbial biomass C and N content (Figure 4b). This result indirectly validates the assumption in current soil COS uptake models that the concentration of CA in soils is approximately proportional

to microbial biomass [24]. Nitrogen fertilisation is known to reduce the amount of microbial biomass [41,54] and especially the relative abundance of mycorrhizal fungi and methanogenic archaea [55] as well as extracellular enzyme activities [41]. Recent studies have also demonstrated that fungal communities can be important drivers of net soil COS fluxes [20,23,56–58]. In particular Sauze et al. [58] showed that decreases in soil fungal abundance correlated significantly with a decrease in the net uptake of COS. Therefore, any decrease in fungal abundance caused by an increase in soil N content would also be consistent with the observed reduction in COS hydrolysis rates in this study. To summarise, N fertilisation has a direct negative effect on CA enzyme activity and an indirect negative effect on the amount of CA in the soil caused by reductions in the microbial community size (biomass) and putatively the community diversity (loss of fungi) upon N fertilisation.

Finally our study highlighted that it may also be important to account for pH when trying to explain the impact of N fertilisation on the variation of COS hydrolysis (Figure 2b). We found that the four alkaline soils that were rich in NO_3^- exhibited higher hydrolysis rates than nitrate-rich acidic soils (Fig. 2b). This suggests that additional mechanisms linked to soil pH must be involved. It has been known that CA activity, at least with regard to CO_2 hydration, is often reduced in acidic buffer solutions as the presence of protons in the solution slows down the re-activation of the active site [59] and also modifies the molecular structure of CA [60,61]. However, most micro-organisms have the ability to buffer their internal pH to a value close to neutral [62], so that soil pH should affect only marginally CA-catalysed COS uptake rates. Another possibility is that, for the same amount of microbial biomass, CA requirements are higher in alkaline environments, thus explaining higher COS hydrolysis rate constants. More detailed kinetic studies on the COS hydrolysis response of different CAs to soil pH alongside comparative metatranscriptomic studies investigating the community differences in CA gene diversity and abundance between low and high pH soils across different N treatments would be needed to provide detailed answers on the interplay between microbial biomass, soil pH and N content. Future genomic studies investigating the variability in soil community composition may also provide insights on whether particular microbial networks or 'keystone' organisms regulate the soil-atmosphere exchange of COS. For example, Meredith et al. [23] recently showed that Actinobacteria and in particular Mycobacteria were highly correlated with the CA activity derived from both CO_2 and COS gas exchange measurements. Altogether, our results already indicate a promising opportunity to predict COS hydrolysis rates from easily measured soil properties including microbial biomass, soil total N and NO_3^- content and pH.

COS production is most closely linked to soil nitrate availability

Our study demonstrated that variations in soil inorganic N content were positively correlated with COS production rates at the European scale, between field plots at the same site and between microcosms manipulated in the lab. We previously showed that the variability of COS production was well correlated with total soil N content [21]. In this study we demonstrated that the labile fraction of N, i.e. NO_3^- and NH_4^+, was strongly related to COS production. First, we observed an increase in COS production with an increase in NO_3^- content across European soils (Figure 2a), but modulated by pH and/or NH_4^+ content, as the effect was significant only at acidic pH (Figure 2a) and high NH_4^+ content (Figure S2b). In addition our lab study also suggested that NO_3^- was the dominant N species affecting COS production rate: the relative change in the COS production was strongly

dependent on the initial N status of the soil prior to fertilisation (Figure 4a), with soils with low initial NO_3^- content responding relatively stronger to the same amount of N addition than those soils already containing high NO_3^- content before fertilisation. This is consistent with the studies described above showing that soils became large COS emitters with N fertilisation [26]. This may also help interpret why agricultural soils frequently exposed to fertilisation might be more likely to emit COS to the atmosphere [19,20,22,23,57].

Mechanisms promoting the production of COS

Currently an intriguing debate exists on the mechanism(s) underlying the production of COS in oxic soils [10]. At 23°C, the magnitude of soil COS production is relatively small compared to the rate of COS uptake observed for the same soil [21]. However, COS production is also more responsive to an increase in temperature than COS uptake [19,21]. Thus, in soils that experience relatively high surface temperatures such as those in Mediterranean regions, COS emissions could rapidly shift soils from being net COS sinks to sources during hot summer months, especially as the rate of COS production in soils was recently shown to be invariable to soil moisture status [21]and may also increase with light [20,27,58,63]. We add to this debate by showing clearly that the rate of COS production is also dependent on soil NO_3^- and/or NH_4^+ content.

Unfortunately, our study was not designed to test specifically whether the exact mechanism driving COS production was abiotic or biotic. Nonetheless, our study does indicate a strong coupling between N and S cycling that might provide some insights on the two main mechanisms recently proposed [10,20]. The first of these mechanisms involves the abiotic or biotic degradation of organic sulphur compounds, in particular the S-containing amino acids cysteine and methionine and to a lesser extent the biomolecule glutathione, that have been identified previously as COS precursors (see Table 1 of Meredith et al. [20] for a review). The amino acids cysteine and methionine are also known to represent a large fraction of the total soil S pool, around 11-31% [64].

Using a novel metatranscriptomics approach, Meredith et al. [23] showed that variations in the COS production rate across a range of soils was strongly correlated to the number of cysteine and methionine synthesis genes present in the soils. In order to reconcile this with our study we would need to hypothesise that the potential amount of amino acids synthesised in the soil community would be dependent on the amount of available inorganic N. This is plausible because microbial biomass S is directly related to microbial biomass C and total soil organic S [65]. Thus in moist active soils with more inorganic N, this would potentially lead to an increased pool of total and S-containing amino acids and subsequently COS precursors that would form the source of either abiotic or biotic COS production. This hypothesis would be entirely consistent with our observations that COS emissions increased with increasing inorganic N content. Future studies should be designed to test this hypothesis directly and determine how the size of this precursor pool changes with N additions.

Microbial decomposition of organic sulfur compounds can also produce COS through a range of different metabolic pathways, including the oxidation of carbon disulphide and thiosulfate [28,29], thiocyanate (SCN^-; [30]) and isothiocyanate [31]. Currently, the SCN^- pathway is the most popular

hypothesis as it has been demonstrated in a number of different microorganisms [30,66–70]. In addition, Kelly et al. [71] showed that bacteria oxidising SCN^- can use NO_3^- as an oxidant for their N supply whilst Broman et al. [72] further demonstrated that the consumption of SCN^- was correlated to NO_3^- consumption in continuous culture bioreactors. This finding may indicate that the removal of NO_3^- limitation (with N fertilisation) in N poor soils might lead to the use of SCN^-. Although NO_3^- is used by many different members of the soil microbial community for processes related exclusively to the N cycle, Kraft et al. [73] showed that a significant portion (25%) of NO_3^- respired in marine microbial communities comes from "cryptic" sulfur cycling. In addition, several bacteria exhibiting significant ecological connections between the C, N and S cycles that can use SCN^- for providing S and N to the cell have been identified. For example, *Tv. Thiocyanodenitrificans* is capable of growth by denitrification with either thiosulfate or SCN^- [74]. *Thiohalophilus thiocyanoxidans* oxidise SCN^- and produce COS [66], and are additionally capable of assimilatory nitrate reduction [67]. Further support for COS production from SCN^- comes from the study of Kantor et al. [69]who demonstrated in bioreactors that microbial communities were able to degrade SCN^- while expressing genes for ammonium oxidation and denitrification. Furthermore, Berben et al. [75] recently showed that *Tv. Thiocyanodenitrificans* contains genes that code SCN^- dehydrogenase in addition to the genes necessary for denitrification. Taken together, this evidence indicates that the oxidation of SCN^- (and therefore the production of COS) and reduction of assimilatory NO_3^- can be potentially realised by the same soil microorganisms. This is supported by the results of Behrendt et al. [76] showing co-variations in the abundance of soil community genes related to N cycling with net COS flux. Therefore, in soils with low NO_3^- content, the conversion of SCN^- to COS could be limited by NO_3^- availability and help explain why the production of COS production is low when NO_3^- concentrations are low and high in nitrate-rich soils as observed in our experiments when NH_4^+ concentrations are similar. Thus, based on our results we could hypothesise that the production of soil COS depends on the ratio of NO_3^- to SCN^-. Future studies combining comparative meta-genomic and -transcriptomic analyses across gradients of soil inorganic N content and COS production will be able to test this hypothesis by exploring whether shifts in the abundances of certain taxa identified above are found, and whether the ratio of genes identified for SCN^- oxidation and nitrate reduction also vary in soil communities. First steps using such an approach were recently made by Meredith et al. [23] to obtain insights on the production rates of COS and genes for S cycling with results lending support for the abiotic degradation of S-containing amino acids. This does not necessarily exclude the production of COS from SCN^- as this precursor can also be produced through the transformation of S-containing amino acids [77]. For example, cyanide ions can react with cysteine in *Bacillus megaterium* to yield SCN^- [77], and also cyanide produced *in vivo* can be converted in part to SCN^- by sulfurtransferase systems present in several microorganisms including the same organisms capable of SCN- oxidation to produce COS [77–79]. Thus again as hypothesised above, an increase in amino acids with N leading to a larger pool of precursors of COS including SCN^- would be compatible with our results.

5. Conclusions

In conclusion, our study reveals a strong and consistent relationship between soil COS exchange and soil nitrogen status. Such fertilisation-induced changes in soil gross COS fluxes are currently not implemented in COS modelling efforts at the global scale [6,12]. However, the most

recent model inversions recognised that the production of COS by agricultural soils should be accounted for explicitly, although it remained unclear why agricultural soils behaved differently from previous studies. Our analysis provides empirical evidence that the important role of N status across soils requires assimilation in the next generation of global COS models. However, capturing the impact of point-source N fertilisation events will prove a modelling challenge to capture the impact on the atmosphere. Finally it is highly likely that increased fertilisation over the past century has reduced the capacity of some soils to take up atmospheric COS. Nonetheless, the results from this study combined with further recent publications in this field have increased our understanding of how COS fluxes vary across space and should facilitate more robust predictions of the exchange of COS between soils and the atmosphere, leading to more robust predictions of global primary productivity using the variability of COS in the atmosphere.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, , Figure S1: Variations in the net COS flux, the COS production rate *P*, the COS uptake rate *k* with soil inorganic N content (NO_3^- + NH_4^+) across 27 soils in Europe. Figure S2: Variations in the net COS flux, the COS production rate *P*, and the COS uptake rate *k* with soil nitrate (NO_3^-) and ammonium (NH_4^+) contents across 27 soils in Europe. Table S1: Characteristics of sites sampled for soil gas exchange. Table S2: Explanatory power of each soil properties for the three COS fluxes across the 27 European soils.

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