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RESEARCH ARTICLE



The reducing effect of aglime on N₂O and CO₂ emissions balance from an acidic soil: A study on intact soil cores

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Abstract

The functioning of the nitrous oxide (N2O) reductase enzyme involved in the last step of denitrification is pH sensitive, with an optimum of 6.8. A solution to mitigate N2O emissions would be to bring soil pH close to neutrality by adding agricultural liming products (aglime). Nevertheless, the influence of aglime on the soil greenhouse gas (GHG) balance (CO₂-N₂O) is a subject of debate, particularly when considering the fate of the carbon (C) derived from carbonates. Our objective was to investigate the results of the effect of calcium carbonate (CaCO₃) aglime on the CO₂-N₂O balance. Sixteen cylinders of undisturbed acidic soil were taken from a sandy loam profile and incubated at 20°C for 107 days in anaerobic conditions (waterfilled pore space >60%). Eight limed treatment cylinders received 1.45 g of aglime on the soil surface (2 t NV ha⁻¹) and 0.08 g of N (100 kg of N ha⁻¹). Eight control treatment cylinders received only 0.08 g of N. N₂O and CO₂ fluxes were measured and converted into CO2 equivalents to perform a GHG balance calculation. Furthermore, soil and leachate properties were measured. Aglime application triggered a reduction of N₂O emissions, probably due to an increase in soil pH at the beginning of the experiment, which would have led to the N₂O reductase activation. High NO₃⁻-N content in the soil may inhibit the high N₂O reduction potential in the limed treatment. CO₂ emissions were unexpectedly lower in the limed treatment. Aglime addition did not enhance C mineralisation, which may be explained by the possible stabilisation of soil organic carbon. A significant 11.3% reduction of GHG emissions was observed in the limed treatment. Overall, our results show that a strategy of liming acidic agricultural soil could be implemented for its potential in GHG mitigation. Nevertheless, future in-depth research is necessary to better understand the fate of the C brought about by aglime.

KEYWORDS

agricultural liming, C stabilisation, denitrification, DOC, GHG balance, NO₃-, pH

1 | INTRODUCTION

Although the fight against global warming continues to evolve and intensify, atmospheric greenhouse gas (GHG) concentrations have never been higher, and have continued to increase more significantly in the last three decades (Ciais et al., 2013). Agriculture is a major contributor to global emissions of GHG (CITEPA, 2019), which include carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N2O). The latter, generally spared by the media spotlight, has a lifetime of about 117 years (Canadell et al., 2021), a high global warming potential 273 times higher than CO₂ (Canadell et al., 2021), and is currently the dominant ozone depleting substance (Ravishankara et al., 2009). In France, agriculture accounts for up to 87% of anthropogenic N₂O emissions, representing nearly 10% of national GHG emissions of anthropogenic origin (CITEPA, 2019).

Agricultural N₂O emissions have been predominately driven by the use of nitrogen (N) fertilisers. Emissions of N₂O from agricultural soils are caused by biotic and abiotic processes. Key biological pathways include nitrification, nitrifier denitrification and denitrification (Stein, 2019). On the one hand, denitrification is considered the major source of N₂O emissions and often associated with the so-called soil N2O emissions hotspots in agriculture (Kravchenko et al., 2017). On the other hand, the last step in denitrification, the reduction of N₂O to dinitrogen (N₂), is the only known terrestrial mechanism allowing the elimination of this gas (Jones et al., 2013). This process of reducing N₂O to N₂ is catalysed by a single enzyme known at present, N_2O reductase, assembled and synthesised by the nosZgene (Scala & Kerkhof, 1999). However, on the global scale, the production of N₂O by soils is greater than its consumption (Schlesinger & Bernhardt, 2013; Wen et al., 2016). Nitrous oxide production and its reduction into N2 and its ratio $(N_2O/[N_2O + N_2])$ depend on soil factors (Wrage et al., 2001), which can be: (i) proximal factors such as the spatial extent of oxygen (O₂) conditions in the soil and N and carbon (C) supply factors that ensure the presence of electron acceptors/donors; and (ii) distal factors that can be biological factors (microbial community abundance), physical factors (soil structure, soil texture or water saturation) or environmental/chemical factors (temperature and pH). These distal factors also affect the proximal factors and vice versa (Groffman et al., 1988). Improving field management practices to control these proximal and distal factors has the potential to mitigate N₂O emissions.

The application of aglime in agriculture is not only beneficial as it alleviates soil acidification problems and greatly benefits soil productivity (Caires et al., 2008), but through the control of soil pH it can play a pivotal role in regulating several soil processes such as organic matter mineralisation,

Highlights

- Significant decreases in CO₂ emissions were observed after adding carbonates to the soil.
- Leached DOC concentrations were lower in the limed treatment, possibly due to C stabilisation.
- High soil NO₃⁻-N content may interfere with N₂O reductase activation.

N transformation, nitrification and denitrification, which, in turn, affect soil N₂O production (Bolan et al., 2011; Shaaban et al., 2014). The effects of aglime application on soil N2O emissions from acidic soils are inherently contradictory, as documented in the literature. Baggs et al. (2010) reported that the application of lime increased C and N mineralisation in acidic soils and therefore enhanced N2O emissions. Aglime application to acidic soils in China showed an elevated concentration of ammonium (NH₄⁺) and nitrate (NO₃⁻), thus considerable releases of N₂O into the atmosphere are due to high nitrification and denitrification rates (Feng et al., 2003). On the contrary, some researchers have documented a decrease in N2O emissions when a liming product was applied to acidic soils (Qu et al., 2014; Shaaban et al., 2015; Weslien et al., 2009). A recent study revealed that N₂O emissions could be decreased through enhanced N2O-reductase activity for soils with a pH ≥6.8 (Hénault et al., 2019). In this latter study, N₂O reduction appeared inefficient at pH <6.4 but was very efficient at pH >6.8 with an intermediate zone of activation, the low soil pH precluding the successful assembly of functional N₂O reductase (Liu et al., 2014).

Dai et al. (2017) estimated that 50% of the world's arable soils are considered acidic (pH <7) and that this percentage continues to increase. Although these figures show the possible high potential of N₂O mitigation through aglime if the targeted pH of 6.8 can be reached and offset increased C and N mineralisation, the question remains regarding the fate of the carbon released from aglime. It has been recognised that the chemical release of CO₂ from aglime can significantly contribute to the CO₂ emissions from agricultural soils. In 2006 and still now, the Intergovernmental Panel on Climate Change suggested that all the carbon in the lime applied is ultimately emitted into the atmosphere in the form of CO_2 . However, this assumption is currently challenged by some authors (e.g., Hamilton et al., 2007) who proposed a biogeochemical theory that suggests that aglime dissolved by weak acids, such as H₂CO₃, whose production is

enhanced by root and microbial respiration, results in the formation of bicarbonate ions (HCO₃⁻) instead of CO₂. Aglime is also considered to improve soil conditions and thus increase microbial respiration and the loss of soil organic carbon (SOC) like CO₂ (e.g., Bertrand et al., 2007). However, aglime effects on SOC have also been called into question by several studies: (i) a better stabilisation of SOC after liming application, in relation to aggregate stability protecting SOC from biodegradation and (ii) changes in biological processes, which enhance SOC mineralisation in response to increased soil pH. The net effect of these processes is not yet well understood (Paradelo et al., 2015).

In addition, the bulk soil pH in the field does not remain constant and will depend on specific management trajectories as well as soil and climatic conditions. Ammoniacal N fertilisation and loss of base cations drive soil acidification (Geisseler & Scow, 2014; Guo et al., 2010) and therefore the targeted soil pH of 6.8 is not only based on aglime application. In addition, increasing soil N concentrations by N fertilisation can interfere with the above proposed liming effect on N_2O mitigation and $N_2O/(N_2O + N_2)$ ratio, as it is well accepted that higher concentrations of NO_3^- in soil inhibit the reduction of N_2O (Baggs et al., 2003; Blackmer & Bremner, 1978; Senbayram et al., 2018).

For all the above reasons, liming is rarely presented as a solution for mitigating soil GHG emissions despite several conclusive effects on N₂O mitigation. If GHG emissions from agricultural soils are to be mitigated, there is a clear need to better understand the role of aglime in the CO₂ and N₂O balance. To date, such information is lacking in the literature with very few studies measuring the liming effect on N₂O and CO₂ emissions simultaneously and certain aspects of the carbon cycle linked to the use of calcium carbonate (CaCO₃) in soil are not fully understood. We conducted a laboratory study on intact soil cylinders, which aimed to examine the effects of N fertilisers combined with the application of aglime on N₂O and CO₂ emissions from an acidic soil in anaerobic conditions. It was hypothesised that: (i) in optimal denitrification conditions (high waterfilled pore space [WFPS] as a proxy of anaerobiosis status, high temperature and high N contents), the application of liming treatment to the soil would result in lowered emissions of N₂O; and (ii) CO₂ emissions could be potentially elevated with the addition of aglime to the soil. Moreover, to address these hypotheses in full, leachates were also analysed to monitor dissolved organic and inorganic carbon (DOC and DIC) concentrations as well as the nitrogen concentrations (NO₃⁻ and NH₄⁺) over the incubation period. Soil physicochemical characteristics were also analysed at the end of the incubation in order to shed further light on the processes underlying the observed evolution of CO₂ and N₂O emissions.

2 | MATERIALS AND METHODS

2.1 | Soil characteristic and experimental design

Intact soil cores (25 cm height, 10 cm diameter) were taken from a farm plot (<2 ha) during the growth of a triticale crop, located in Fourche Village, Morvan region, France (47°15′38.369" N; 4°11′18.952" E). The soil had a sandy loam texture (Table 1) and was classified as a Brunisol according to the NGI (National Geographic Institute). Details on the soil texture are given in Table 1. The initial soil analysis was performed on one composite made from four subsamples taken from each pit (see below), sieved at 5 mm and well homogenised. This site was chosen for its low pH (5.7 \pm 0.1) and low capacity to reduce N₂O into N₂ (index >50, Table 1). The soil's capacity to reduce N₂O was determined using soil samples collected in the field and a laboratory test (ISO/TS 20131-2) derived from the laboratory protocol used by Hénault et al. (2001). This provided quantitative indicators (r_{max} ranging from 0 to 1 and INDEX ranging from 0 to 120) with rules of interpretation (the higher the r_{max} and INDEX are, the lower the capacity of the soil to reduce N₂O is).

A total of 16 intact soil cores (8 replicates per treatment) were sampled at the west part of the field where an area of 250 m² (50 m \times 5 m) was delimited. The cores were obtained by driving steel cylinders manually into the soil and removing them (Hénault & Germon, 2000) on March 25, 2021. The first 20 cm of the soil surface were taken into consideration since N2O dynamics (production and reduction) is known to be greater than in the subsurface soil (Hu et al., 2020). The first 5 cm were left free to avoid the effect of soil compaction while driving the cylinders into the soil. In the delimited area, the cylinders were organised in lines of four cylinders (two per treatment in each line) spaced 10 cm apart, and each line of four cylinders spaced by 10 metres. Four pits (30 cm deep, 50 cm wide) were dug around the cylinders in order to remove them. It was hypothesised that spatial dependence would not be observed at this scale (Mathieu et al., 2006). The cylinders were then placed in a temperature control chamber (20°C) throughout the experiment. The steel cylinders were closed with an airtight PVC cover (top and bottom) fitted with septa for gas samplings, leachate samplings and air flow, as described in Figure 1. The steel cylinder had a grid and a nylon mesh placed over the bottom of the ring to prevent soil egress.

The experimental design consisted of two treatments: Control (N fertiliser only) and Limed (N fertiliser and aglime) replicated eight times. The mineral N fertiliser (36% N), composed of 60% urea and 40% ammonium sulphate, used in both treatments was applied on top of the cylinder



TABLE 1 Physico-chemical properties of the Fourche village soil for the top soil (0–20 cm).

<u> </u>					
Property	Quantity				
Texture ^a	Sandy loam				
Clay/% ^a	14.7				
Silt/% ^a	24.5				
Sand/% ^a	60.8				
Bulk density/g cm ^{-3b}	1.52 ± 0.01 (s.e.m)				
Gravimetric water content at field capacity (-33 kPa)/g g ^{-1c}	0.23				
Soil pH	$5.7 \pm 0.08 (\text{s.e.m})$				
Total carbonate/% ^a	< 0.5				
Organic matter content/g kg ^{-1a}	42.8				
Total carbon organic/g kg ^{-1a}	24.7				
Cation exchange capacity ^a	Low _{metson} (130 meq kg ⁻¹)				
r_{max}	1				
INDEX	183				

^aAnalyses undertaken by the SADEF laboratory.

soil at a rate of 33 mg N kg⁻¹ soil (i.e., 0.22 g of fertiliser per cylinder, 100 kg ha⁻¹). The amount of N added to the soil emulated the quantity of N fertiliser typically applied under winter cereal crops in the sampling zone (Dreal, 2017).

The aglime used for the limed treatment (Calcimer[®]) was a CaCO₃ aglime of marine origin marketed by Timac Agro with a neutralising value (NV) of 40.

The quantity of aglime applied was calculated to target a pH of 6.8 and according to the nonlinear model defined by Rémy and Marin Laflèche (1974) as follows:

$$\begin{aligned} \text{BEB}_{\text{red}} &= \left[0.055 \times (\text{CLAY} + 5 \times \text{OM}) \times \left(e^{\frac{\text{pHs}}{1.5}} - e^{\frac{\text{pHa}}{1.5}}\right)\right] \\ &\times \text{P.T.F} / 1000 \end{aligned}$$

with BEB_{red} being the neutralizing base needs in units of NV/ha, CLAY is the clay content in ‰, OM is the organic matter content (‰), pHs is the targeted pH, pHa the current pH value and P.T.F is the weight of fine soil (<2 mm) in tonne/ha (density × soil depth in cm x% of fine soil). The quantity of aglime (ton ha⁻¹) to apply was calculated as follows: BRB_{red} × 100/NV_{aglime}, thus, 0.25 mg NV g⁻¹ soil of aglime was applied per cylinder (2 t NV ha⁻¹). This entailed adding 0.075 mg C g⁻¹ soil, representing 0.3% of the C pool in the soil, the remaining 99.7% of C coming from the soil organic carbon (SOC).

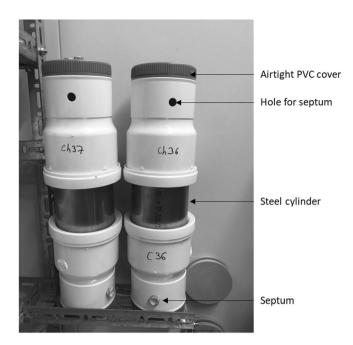


FIGURE 1 Picture and legend of the airtight cylinder system used to determine gas emissions and leachate compositions.

The liming product and N fertiliser were applied manually on April 14, 2021 (Day 0) on top of the soil surface.

Soil WFPS was used to characterise soil aerobiosis/ anaerobiosis status, with anaerobiosis, and consequently denitrification, starting at a WFPS equal to 60% (Bateman & Baggs, 2005) but also depending on O_2 exchanges. Expressed as a percentage, WFPS is the ratio of volumetric soil water content to total soil porosity (estimated at 43% for the cylinders soil). Before aglime addition (Day -1), the average WFPS was 53% for the 16 cylinders. Water was applied punctually (between the sampling points) on each soil cylinder in order to gradually increase the soil WFPS to progressively reach the WFPS at field capacity (FC) equal to 82% and then until porosity saturation (WFPS = 100%), ensuring that denitrification could occur.

2.2 | Sampling procedure

Sampling points were taken the day before applying the treatments (Day -1) and over a 107-day period, for a total of 15 discontinuous gas measurements. First, the leachate was collected at the bottom of each cylinder with 100 mL flasks. WFPS was then adjusted at the top using a dispenser to apply the same amount of water for each cylinder up to a maximum of 100 mL. In total, 780 mL of water was added to each cylinder over the incubation period, or the equivalent of 100 mm rainfall. This rainfall regime is consistent with the relative rainfall during the season of testing in Morvan region, where

^bMeasured using the soil initial water characteristics of each pit and soil mass and volume of each cylinder (Jury et al., 1991).

^cMeasured on a ceramic pressure plate extractor, PPE (Soil moisture Equipment Corp., California, USA) for matric potentials ranging from 100 to 1500 kPa (ISO/NF 11274).

around 80 mm of rain falls per month with an average rainfall higher than 100 mm for May and December months (Meteoblue).

After adjusting WFPS, all the septa (6 per cylinder) were reinstalled and once the airtightness was restored, 5 mL of krypton (Kr) (Alphagaz, France) was injected into the cylinders to evaluate the total gas volume in each cylinder and verify that there was no leakage. The atmosphere of each cylinder was sampled in pre-vacuumed 10 mL vials four times over a 3-hour period (0, 1, 2 and 3 h) with a 12 mL syringe connected to a 26G hypodermic needle. Between the first and the second gas samples, the cylinders' weights were recorded to determine the moisture content. At the end of the 3 h, the cylinders were reopened, and the 2 septa removed from the upper PVC part (Figure 1).

2.3 | Nitrous oxide and carbon dioxide analyses

Nitrous oxide and CO_2 concentrations were analysed on a Trace 1310 series gas chromatograph (GC, Thermo Fisher Scientific) coupled with an autosampler (HSS 39–50 SRA Instruments) and on an Agilent 990 microGC, respectively. As for the CO_2 , the Kr concentrations were also analysed on an Agilent 990 microGC. The integrity of the gas concentrations in the vials was tested using sets of standards, premade by diluting pure N_2O and pure CO_2 in the experimental conditions and validated with certified standard bottles. The change in the quantity of N_2O or CO_2 produced (μ g N_2O –Ng⁻¹ dry soil and μ g CO_2 –Cg⁻¹ dry soil, respectively) over time was used to calculate N_2O and CO_2 fluxes (gN– N_2O ha⁻¹ day⁻¹, kg C– CO_2 ha⁻¹ day⁻¹) with the quantity measured as follows (example given for the quantity of N_2O):

$$\begin{aligned} \mathbf{Q}_{\mathrm{N_2O}} &= \left\{ [\mathrm{N_2O}] \times \frac{V_{\mathrm{gaz}}}{V_{\mathrm{mol}}} + n_{\mathrm{water}} \times [\mathrm{N_2O}] \times K \times P_{\mathrm{atm}} \right\} \\ &\times 28 / m_{\mathrm{dry \, soil}} \times 10^6 \end{aligned}$$

where, $Q_{\rm N2O}$ is the quantity of $N_2{\rm O}$ produced by soil unit in $N_2{\rm O}$ –N $\mu{\rm g}$ ${\rm g}^{-1}$ of dry soil, $[N_2{\rm O}]$ is the $N_2{\rm O}$ concentration in the flask atmosphere in $1\,1^{-1}$, $V_{\rm gaz}$ is the gaseous volume in the bottle in ${\rm l}$, $V_{\rm mol}$ is the molar volume at 20°C and atmospheric pressure in ${\rm l}$, $n_{\rm water}$ is the number of mol of water in each bottle, K is the solubility constant in water of $N_2{\rm O}$; $K=0.48.10^{-6}$ mol mol $^{-1}$ hPa $^{-1}$ at 20°C, $P_{\rm atm}$ is the atmospheric pressure in hPa, $m_{\rm drysoil}$ is the mass of dry soil in g.

The IPCC calculation assumes that the addition rate of aglime is in near equilibrium with the consumption of aglime applied in the previous year. In other words, all the C from the aglime is assumed to be consumed over a

year. Then, the emissions associated with liming can be estimated using the amount of aglime applied. The calculation of the C emissions from liming (CEL) over a year was obtained by multiplying the amount of lime with a C conversion factor, 0.12 in the case of $CaCO_3$ based on the molar masses of C and $CaCO_3$. Thus, the CEL was estimated at 0.625 kg C ha⁻¹ day⁻¹.

Finally, gas fluxes are specified in CO_2 equivalent considering the global warming potential of each gas in order to perform a N_2O and CO_2 balance calculation. The limed treatment was then compared with the control to measure the abatement as follows:

Abatement (%) =
$$\frac{(Flux_{control} - Flux_{limed})}{Flux_{control}} \times 100$$

The flux averages over the incubation period were taken for the abatement calculation. No cumulative data were determined for this study due to the discontinuous lag time between sampling points, thus linear interpolation between sampling dates could not be assessed. Therefore, the C and N budgets for the entirety of the experiment could not be determined and would have required continuous CO_2 , CH_4 and N_2O measurements.

2.4 | Leachate analyses

The weight of the leachates was recorded, pH measurements were performed with a pH meter (Mettler Toledo SevenCompact) and NO_3^- -N and NH_4^+ -N concentrations were measured using the spectrophotometric method (Yang et al., 1998). The detection limit was 0.01 mg N kg $^{-1}$ soil. When the volume was sufficient, leachates were then filtered (Whatman GD/X 25) and subsamples were taken and analysed for dissolved inorganic or organic carbon (DIC and DOC) on an Elemental TOC analyser. As it was not possible to collect the soil from the cylinder during the incubation period without jeopardizing the undisturbed measuring system, the leachates were analysed from the perspective to inform on the soil cylinder profile dynamic.

2.5 | Destruction day analyses

At the end of the incubation period, the soil of each cylinder was extruded separately, sieved at 5 mm and kept in Ziploc[®] plastic bags. A 10 g subsample was taken to determine the gravimetric water content at 105°C for 24 h. Another 10 g subsample was shaken for 1 h with 50 mL of DI water to measure the soil pH (NF ISO 10390 Mai 2005/X31-117). The equivalent of 10 g of dry soil was

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extracted with 50 mL of potassium sulphate (0.5 M K_2SO_4), and shaken for 1 h to determine inorganic-N concentrations. After filtering (Whatman 42), the extracts were analysed for NO_3^- -N and NH_4^+ -N concentrations using the spectrophotometric method (Yang et al., 1998). Subsamples were taken to determine the soil capacity of each cylinder to reduce N_2O into N_2 using the laboratory test (ISO/TS 20131–2) previously described.

2.6 | Statistical analyses

The effect of limed treatment on CO₂ and N₂O emissions over time was statistically analysed by two-way repeated measures analyses of variance (ANOVA), with limed treatments (L), time (T) and the interaction as factors. The residuals were tested visually for normality, distribution and homoscedasticity using the QQ plot, Residuals vs. Fitted plot and the Scale-location plot, respectively. After the hypotheses on the residuals were validated, the results of the ANOVA were processed. If the hypothesis regarding residuals were refuted, the best fit transformation was applied using the "powerTransformation" function in the car package (John Fox and Sanford Weisber, 2019) as a decision tool. This function uses the maximum likelihood-like approach of Box and Cox (1964) to select a transformation of a univariate or multivariate response for normality, linearity and/or constant variance. If this proved insufficient, appropriate nonparametric tests were then applied on the response variables, depending on the number of factors involved (e.g., Kruskal–Wallis test or a two-way repeated measures ANOVA with a rank transformation on the variable; Higgins et al., 1990).

Other statistical analyses were performed on the additional response variables measured in this study (e.g., DIC and DOC concentrations in the leachates) with often the same three factors involved: "limed treatment", "time" and the "limed treatment*time" interactions following the same procedure as described above. More details on the statistical hypothesis, tests and transformations can be found in Table S1.

For each statistical analysis, the level of p < 0.05 was considered statistically significant. In the case of a significant interaction (limed treatment*time), comparisons of the means relative to the modalities of one factor (limed treatment) were made separately for each of the modalities of the other factor (time) using Tukey's post hoc test to test for significant differences between means at the 5% probability level.

All the statistical analyses were carried out using R version 4.1.2 (R core team, 2018). The graphic representations of the data were made using the ggplot2 package

(Wickham, 2016). All the graphics present the averages of the replicates ($n \le 8$) and, in the absence of any notification, the error bars are the standard error of the mean (SEM) and calculated as follows:

$$SEM = \frac{\sigma}{\sqrt{n}}$$

with σ being the standard deviation of the mean and n is the sample size.

In the body of the text, SEM can be found as the uncertainty of the mean (mean \pm SEM).

3 | RESULTS

3.1 | Soil anaerobic conditions and greenhouse gas emissions over the incubation period

After liming application, WFPS stayed over 60% for both treatments (Figure 2). The WFPS trends reflected water supplies with a gradual increase in order to reach field capacity (82%) until day 34. Thereafter, the WFPS kept increasing significantly until it reached a maximum on day 73 for both treatments, with 96.9% for the control and 97.3% for the limed treatment. At the end of the incubation period, the WFPS for both treatments were under FC.

There was no significant difference between treatments on WFPS (Table 3), with an average of 75.5% for the control treatment and 74% for the limed treatment.

Comparing the N_2O fluxes with variations in WFPS suggests that soil N_2O fluxes for both treatments tended to increase as the soil WFPS increased over time (Figure 2). There was a significant time effect on the N_2O fluxes. When looking at the soil N_2O fluxes before (day -1) and after (\geq day 1) the treatments were applied, it should be noted that the N_2O fluxes started to become substantially higher than the N_2O fluxes on day -1, compared with all days \geq 8. The highest N_2O -N fluxes occurred on day 79 for both treatments (Figure 2), following soil drainage (on average, the WFPS decreased by 6.5% between day 72 and day 79 for both treatments), reaching 244.2 ± 70.6 g N ha $^{-1}$ day $^{-1}$ for the control treatment and 250.3 ± 65.1 g N ha $^{-1}$ day $^{-1}$ for the limed treatment.

Over time, the mean N_2O fluxes were 10 times lower for the limed treatment and only 4 times lower for the control treatment (Figure 2). On day 48, the N_2O emissions for the limed treatment were significantly lower than for the control. Overall, the effect of limed treatment was not significant (Table 2), even if noticeable lower N_2O emissions for the limed treatment (41.9 g N ha⁻¹ day⁻¹ in average)

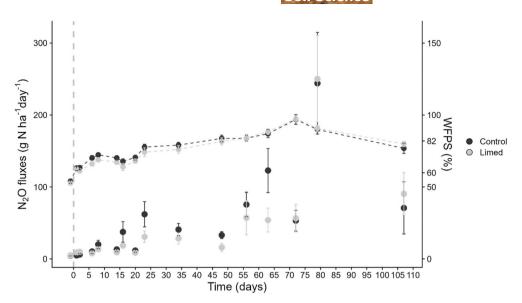


TABLE 2 Two-way repeated measures anova results of factors on the main variables.

		-								
	N ₂ O (after <i>log</i> transformation)					CO ₂				
Factor	Df	Sum Sq	Mean sq	F value	p value	Df	Sum Sq	Mean sq	F value	p value
Lime (L)	1	4.85	4.847	0.76	ns	1	461.7	461.7	4.784	*
Residuals	14	89.29	6.378			14	1351.3	96.5		
Time (T)	14	212.06	15.147	35.147	***	14	6092	435.1	45.17	***
$L\times T$	14	17.37	1.240	2.878	***	14	429	30.6	3.18	***
Residuals	196	84.47	0.431			196	1888	9.6		
Factor	CO_2 eq emissions ($CO_2 + N_2O$) (rank)									
Lime (L)	1	74,131	74,131	4.633	*					
Residuals	14	224,018	16,001							
Time (T)	14	334,192	23,871	10.805	***					
$L\times T$	14	86,624	6187	2.801	***					
Residuals	196	433,009	2209							

Note: Rank referred to a two-way repeated measures ANOVA with a rank transformation on the variable. Abbreviation: ns: not significant.

compared with the control (51.2 g N $\mathrm{ha}^{-1}\,\mathrm{day}^{-1}$) were observed.

Despite an equivalent baseline N_2O-N fluxes between treatments, the baseline CO_2-C fluxes (day -1 on Figure 3) were different between the limed and control treatments, with an average of 13.9 kg C ha⁻¹ day⁻¹ and 10.1 kg C ha⁻¹ day⁻¹, respectively. With the addition of aglime, this trend was quickly reversed with higher CO_2-C fluxes for the control treatment compared with the limed treatment on day 1 (Figure 3). The CO_2-C fluxes increased significantly after adding both amendments from

 10.1 ± 1 to 28.2 ± 1 kg C ha $^{-1}$ day $^{-1}$ (for a difference of 18.1 kg C ha $^{-1}$ day $^{-1}$) and 13.9 ± 0.8 to 25 ± 1.5 kg CO $_2$ –C ha $^{-1}$ day $^{-1}$ (for a difference of 11.1 kg C ha $^{-1}$ day $^{-1}$) for the control and limed treatments, respectively (Figure 3). Then, the CO $_2$ –C fluxes decreased over time to reach a minimum on day 79 for both treatments, 8.22 ± 0.9 kg C ha $^{-1}$ day $^{-1}$ for the control treatment and 7.14 ± 0.7 kg C ha $^{-1}$ day $^{-1}$ for the limed treatment.

After adding both amendments, the mean CO₂ fluxes were lower for the limed treatment compared with the control treatment on 12 occasions with the reverse

^{***} $p \le 0.001$.

^{**} $p \le 0.01$.

^{*} $p \le 0.5$.

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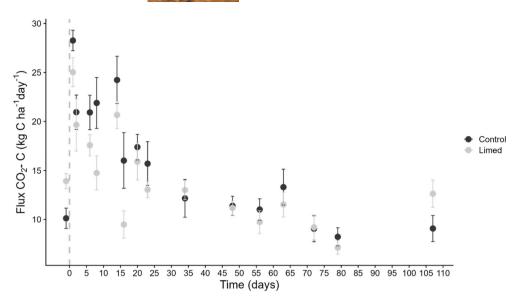


FIGURE 3 Evolution of mean daily CO_2 fluxes over time for the control (N alone) and limed (N + aglime) treatments. Both N and aglime were applied on day 0, indicated by the vertical grey line. Error bars = s.e.m. n = 8.

TABLE 3 Two-way repeated measures ANOVA results of factors on the additional variables.

	pH le	pH _{leachate} (rank)					WFPS				
Factor	Df	Sum Sq	Mean sq	F value	p value	Df	Sum Sq	Mean sq	F value	p value	
Lime (L)	1	327,443	327,443	166.2	***	1	131	131.4	0.389	ns	
Residuals	14	14	2758	1971		14	4731	337.9			
Time (T)	11	100,932	9176	12.941	***	14	24,989	1784.9	123.819	***	
$L\times T$	11	24,398	2218	3.128	***	14	276	19.7	1.366	ns	
Residuals	154	109,196	709			196	2825	14.4			
	NO ₃ - _{leachate} (rank)						NH ₄ ⁺ _{leachate} (rank)				
Lime (L)	1	5	4.7	0.003	ns	1	42,483	42,483	19.33	***	
Residuals	14	24,648	1760.6			14	30,771	2198			
Time (T)	11	413,635	37,603	47.538	***	11	394,262	35,842	53.635	***	
$L\times T$	11	29,694	2699	3.413	***	11	14,809	1346	2.015	ns	
Residuals	154	121,815	791			154	102,912	668			
	DOC _{leachate} (rank)					DIC _{leachate} (rank)					
Lime (L)	1	99,634	99,634	14.67	**	1	13,172	13,172	2.26	ns	
Residuals	14	95,073	6791			14	81,583	5827			
Time (T)	4	601,530	150,382	27.863	***	4	288,705	72,176	11.06	***	
$L\times T$	4	49,521	12,380	2.294	ns	4	399,632	99,908	15.32	***	
Residuals	56	302,244	5397			56	365,277	6523			

Note: Rank referred to a two-way repeated measures ANOVA with a rank transformation on the variable. Abbreviation: ns, not significant.

occurring only 3 times (Figure 3). A significant difference between treatments over time (Table 2) was observed with unexpected lower CO_2 -C fluxes for the limed treatment (13.5 kg C ha⁻¹ day⁻¹) compared with the control treatment

 $(15.2 \text{ kg C ha}^{-1} \text{ day}^{-1})$ and lower than the expected $\text{CO}_2\text{-C}$ fluxes based on the CEL calculation (0.625 kg C ha⁻¹ day⁻¹). At the end of the incubation period, the $\text{CO}_2\text{-C}$ fluxes were, once again, higher for the limed treatment compared with

^{***} $p \le 0.001$.

^{**} $p \le 0.01$.

 $[*]p \le 0.5.$

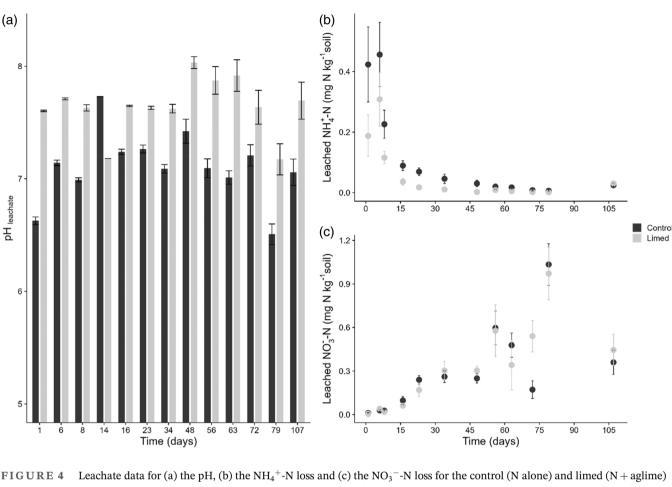


FIGURE 4 Leachate data for (a) the pH, (b) the NH_4^+ -N loss and (c) the NO_3^- -N loss for the control (N alone) and limed (N + aglime) treatments applied on day 0. Error bars = s.e.m. The sample number varied from 3 to 8 depending on the date due of the available leachate volume.

the control treatment, with values close to the initial baseline (11.4 and 9.1 kg C ha⁻¹ day⁻¹, respectively).

Under the conditions of this experiment, the addition of liming product reduced the N_2O and CO_2 emissions balance by 11.3% on average, based on 15 discontinuous gas measurements after 107 days of incubation, compared with the control treatment, with significantly higher emissions for the control treatment with 76.7 kg eq CO_2 ha⁻¹ day⁻¹ than the limed treatment, with 68 kg eq CO_2 ha⁻¹ day⁻¹ emitted (Table 2).

3.2 | Leachate composition and properties over time

The pH measured in the leachates was higher than the initial soil pH (5.7) from day 1 and throughout the incubation period even for the control treatment (Figure 4a). On average, the pH in the leachates was significantly higher (Table 3) in the limed treatment (7.5) compared with the control treatment (6.9) and this liming effect lasted over the whole incubation period (Figure 4a).

Leachate NH_4^+-N concentrations were significantly higher (Table 3) at the beginning of the incubation and decreased over time (Figure 4b). The highest NH_4^+-N leached for both treatments was obtained on day 6 and was equal to a loss per cylinder of 0.45 ± 0.1 and 0.31 ± 0.1 mg of NH_4^+-N kg $^{-1}$ of soil for the control and limed treatments, respectively. Under the limed treatments NH_4^+-N losses were significantly lower compared with the control over time (Table 3). Under both treatments, NH_4^+-N leaching effectively ceased after day 48 (Figure 4b) and 1.41 and 0.72 mg of NH_4^+-N kg $^{-1}$ of soil in total were lost during the incubation period for the control and limed treatments, respectively.

In parallel, the quantities of NO_3^--N leached increased significantly for both treatments over time until reaching a maximum on day 79 $1.03\pm0.1\,\mathrm{mg}\,NO_3^--N\,\mathrm{kg}^{-1}$ of soil and $0.97\pm0.2\,\mathrm{mg}\,NO_3^--N\,\mathrm{kg}^{-1}$ lost from the soil for the control and limed treatments, respectively (Figure 4c). On day 107 (last day of incubation), the NO_3^--N concentration in the leachates for both treatments dropped to approximately $0.4\,\mathrm{mg}$ of NO_3^--N lost (Figure 4c). In total, 3.55 and $3.78\,\mathrm{mg}$ of $NO_3^--N\,\mathrm{kg}^{-1}$ of

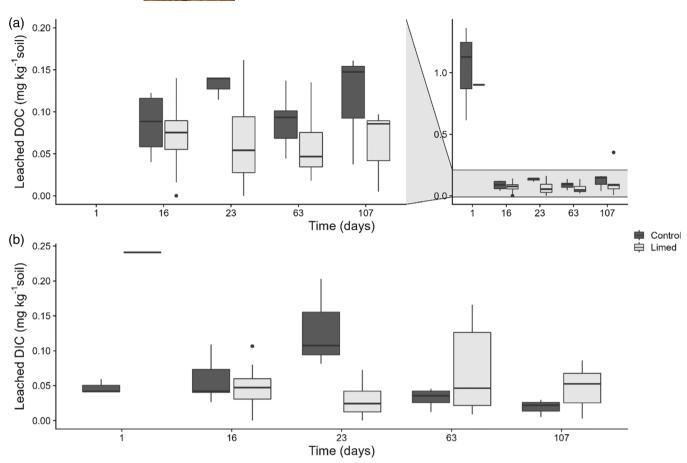


FIGURE 5 Boxplots showing the distribution of (a) the organic (DOC) and (b) the inorganic (DOC) carbon quantities leached over time. Both treatments, the control (N alone) and limed (N + aglime), were applied on day 0. The sample number varied from 3 to 8 depending on the date due to the available leachate volume.

soil were lost during the incubation period for the control and limed treatments, respectively. There was no significant effect of the limed treatment over the control treatment (Table 3). The quantities of NO_3^- -N leached were several times higher than the quantities of NH_4^+ -N leached.

The analyses for dissolved C (organic, DOC or inorganic, DIC) were performed only for days 1, 16, 23, 63 and 107 as the volume was sufficient and the N_2O emissions peaked on those days (Figure 2). The first thing to note was the considerable variability between replicates, notably for the quantity of DIC leached per cylinder with extended boxplots (Figure 5). After applying the treatments, the quantity of DOC lost per cylinder in the leachate was high (day 1, Figure 5a) for both treatments with a loss >0.9 mg kg $^{-1}$ of soil after which it dropped significantly below 0.2 mg kg $^{-1}$ soil for the remaining days (days 16, 23, 63 and 107). In the limed treatment, the quantity of DOC lost per cylinder in the leachate was significantly lower than the quantity of DOC lost in the control treatment cylinders over the incubation period (Table 3).

The pattern of results observed for the DIC quantities in the leachate was less clear; with no significant difference between treatments over time but with a significant effect of the "Time \times Limed treatment" interaction (Table 3). For the control treatment, the DIC quantities lost per cylinder in the leachates remained <0.25 mg kg $^{-1}$, increased up to day 23 (Figure 5b) to reach a maximum of 0.13 mg on average, and then decreased on days 63 and 107 to a minimum of 0.02 mg kg $^{-1}$ of soil. For the limed treatment, the maximum DIC quantity was reached on day 1 (Figure 5b) with, on average, 0.24 mg of DIC kg $^{-1}$ of soil lost per cylinder (almost four times lower than the magnitude of DOC lost on the same day). For the remaining days, the quantities of DIC lost per cylinder for the limed treatment remained low (around 0.05 mg kg $^{-1}$).

3.3 | Soil properties at the end of the incubation period

Soil pH at the end of the experiment varied with treatments: the average soil pH for the limed treatment (5.7) was significantly higher than the average soil pH for the control treatment (5.3). As the initial soil pH

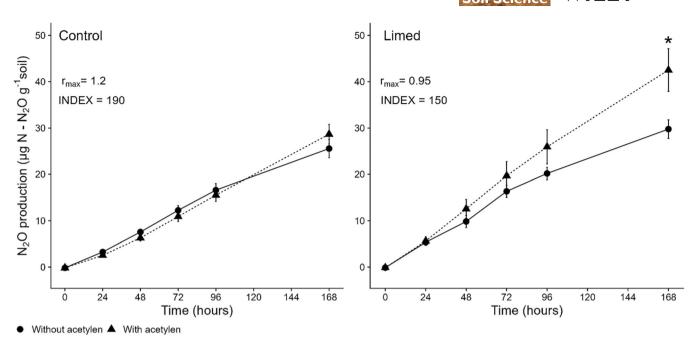


FIGURE 6 Kinetics of soil denitrification from the limed and control treatments, placed in anaerobic conditions for 168 h with nitrate addition, with and without acetylene. r_{max} is the maximum ratio of the accumulated N_2O and the INDEX is the product of r_{max} and t, with t being the time over which N_2O accumulates in the bottles during incubation without acetylene (when the production rate is higher than that of consumption). *stands for a significant difference (p < 0.05) between the two acetylene treatments (with or without).

was 5.7, a significant decrease ($p \le 0.05$) in soil pH was then observed in the control treatment at the end of the experiment. However, despite the addition of liming product, the soil pH at the end of the experiment was acidic (5.7) and not significantly higher than the initial soil pH.

From the soil subsamples collected on the day of destruction per cylinder, the average NO_3^--N concentrations for the control and limed treatments were 11.1 ± 0.9 and $9.56\pm3\,\mathrm{mg}\ NO_3^--N\,\mathrm{kg}^{-1}$ of dry soil, respectively. There was no significant difference between treatments and the measured soil concentration corresponded to one third of the N fertiliser applied (33 mg N kg $^{-1}$ soil). The concentrations in NH_4^+-N at the end of the experiment in the soil were under our detection limit.

In the control treatment, the soil's capacity to reduce N_2O into N_2 at the end of the experiment was similar to that of bare soil at the beginning with a high INDEX of 190 (INDEX = 183 on Day -1, Table 1) and a low percentage of N_2O consumption at the end of the 168 h anaerobiosis incubation (12.3%). The presence or absence of acetylene did not have any effect on N_2O production over incubation time (168 h) for the control treatment (Figure 6). Despite a significant difference observed in the limed treatment at the end of incubation ($p \le 0.05$) with

less N_2O production in the absence of acetylene due to a potential activation of N_2O reductase, both indicators r_{max} and INDEX were high and suggest the soil's capacity to reduce N_2O was poor.

Together, pH and N_2O reduction indicators reveal that the liming effect stopped after 107 incubation days at $20^{\circ}C$. The soil denitrification kinetics was measured only before and after incubation. Consequently, and despite showing an evolution of the denitrification kinetics in the limed treatment, these measures cannot be taken as a correct representation of the N_2O reductase activity during incubation for both treatments.

4 | DISCUSSION

As a contribution to evaluate the extent to which liming acidic soils could be used as a lever for mitigating soil N_2O emission, this study investigated and contradicted the main criticism generally made against this potential lever, that is, the increase in CO_2 when lime neutralises soil acidity (Goulding, 2016). The balance between N_2O and CO_2 emissions must therefore be reported in national greenhouse gas inventories (De Klein et al., 2006) and this study helps to set the stage for further laboratory and field investigations on the effect of liming application on the GHG balance.

4.1 | Evolution of N₂O fluxes after applying liming products

Soil N2O flux averages measured for both treatments were consistent with fluxes previously observed in situ (Stehfest & Bouwman, 2006). The significant increase in leached NO₃ over the incubation period in both treatments, together with the decrease in NH₄⁺ suggest that nitrification took place (Stein & Klotz, 2011). An increase in NO₃⁻ in the cylinder soil led to an increase in N outputs (N₂O and N₂) through denitrification (e.g., Bhandral et al., 2007). The observed temporal variation in the magnitude of N₂O fluxes can be explained by different factors affecting the denitrification rate and the $N_2/(N_2O + N_2)$ ratio. This study was performed in conditions favourable for the denitrification process to occur (high WFPS as a proxy of anaerobiosis status, high temperature and high N contents). Soil oxygen supply acts as the primary driver for initiating denitrification (Rohe et al., 2021). The increase in N2O fluxes for both treatments occurred as the increasingly anaerobic conditions, as defined by an increase in WFPS (≥70% after day 6, Figure 2), were conducive to the denitrification process (Butterbach-Bahl et al., 2013). During this process, the N₂O formed can be reduced to N₂ or escape into the atmosphere (Braker & Conrad, 2011; Hochstein & Tomlinson, 1988; Rohe et al., 2021). The physical conditions permitting the retention of N₂O in isolated air pockets were created in this study and thus provided more time for N₂O to be denitrified to N₂ (Klefoth et al., 2014).

With noticeable lower N2O emissions over the incubation period for the limed treatment compared with the control, this study is consistent with previous studies demonstrating the mitigating effect of soil liming application on N₂O emissions (Shaaban et al., 2015; Weslien et al., 2009). The results of the experiments performed at different scales, from the enzyme to the field, concurred with the assumption that increasing soil pH by the application of aglime favours the complete denitrification process to occur, implying the reduction of N₂O into N₂. Nitrous oxide reductase is the sole enzyme of the denitrification process, which reduces N2O into N2 and its activity is regulated, amongst other soil factors, by soil pH (Wrage et al., 2001). This activity is very slow in soils with a pH below 6.4, and it starts occurring at a pH between 6.4 and 6.8 to become fully functional at pH >6.8 (Hénault et al., 2019), with acid soil pH cancelling the successful assembly of functional N2O reductase (Liu et al., 2014).

Despite the absence of direct evidence on the increased soil pH during the incubation period for the limed treatments, because no soil sample was taken, it can be hypothesised that it evolved. The application of

liming materials to soils is known to increase soil pH rapidly, within a week, after application (Holland et al., 2018; Osei, 1995; Shaaban et al., 2018), suggesting a rapid pH increase in the limed soil cylinders. This increased pH could have then decreased over time to return to the initial value, as observed at the end of the incubation period. The pH of soils receiving ammoniacal nitrogen fertilisation and subject to a loss of base cations was observed to decrease (Geisseler & Scow, 2014; Guo et al., 2010). Significantly higher pH in the leachate within the limed treatment compared with the control treatment highlighted the fact that more exchangeable cations are washed from the soil during leaching, suggesting that some of the aglime applied was leached in our experimental system. The assumption that the application of CaCO3 in our experiment rapidly increased soil pH, making functional the N₂O reductase, is consistent with the lower N2O emissions observed on limed soil cores due to enhanced N₂O reduction.

Moreover, it is also noteworthy that the amount of NH₄⁺-N leached (Figure 4b) was significantly lower for the limed treatment over the incubation period. This experimental result suggests a faster nitrification in the limed treatment, which theoretically could have resulted in more N₂O emissions by nitrification (during abiotic NH₂OH decomposition and as a by-product of nitrification of NH₃ or/and by the nitrifier denitrification process; Stein, 2019). As no increase of N₂O emissions in the limed treatment was found, it could be suggested that the vigorous O₂ consumption by nitrification (2 mol O₂ are consumed for each mol NH₃ oxidised to NO₃⁻) may have induced coupled nitrification-denitrification (Wrage et al., 2001) with the denitrifiers using the nitrification products (Wrage-Mönnig et al., 2018). The N₂O/(N₂O $+N_2$) stoichiometry of the coupled nitrificationdenitrification will therefore also be affected by soil pH with better reduction of N₂O emissions in the limed treatment (Nadeem et al., 2020).

The effect of liming application on N_2O emissions was not significantly reproducible at every time point, as it would have been expected under similar denitrification conditions. Changes in soil pH combined with elevated soil NO_3^- -N concentrations could be one of the explanations as they may have prevented N_2O reduction, thus increasing the $N_2O/(N_2O+N_2)$ ratio. The electron acceptor, NO_3^- , is usually preferred over N_2O as a terminal electron acceptor and N_2O can escape from the soil whenever NO_3^- supply is greater than the falling demand of denitrifiers (Baggs et al., 2003; Blackmer & Bremner, 1978; Senbayram et al., 2018). Recently, this effect of soil NO_3^- -N concentration was shown to override the effect of liming with respect to the $N_2O/(N_2O+N_2)$ ratio in a sandy cropping soil: high concentrations

of NO₃⁻-N (45 mg N kg⁻¹ soil) almost completely inhibited N₂O reduction (Senbayram et al., 2019). Hence, the small difference between treatments (control and limed) observed in the current study compared with Hénault et al. (2019) and Shaaban et al. (2019) may have been a partial consequence of the relatively high soil NO₃⁻-N concentrations increasing the $N_2O/(N_2O + N_2)$ ratio. The reduction of pH in the limed treatment could have also been a factor, with the N2O reductase potentially becoming less functional once the pH falls below 6.8. This raise the question of the response of the N₂O reductase over time after the application of liming products and future research is needed to continue examining the mechanism underlying the factors altering the $N_2O/(N_2O + N_2)$ ratio, given the effects of C and N supply and soil O2 status on denitrification.

These results are inherent of this experimental design (discontinuous sampling points) and will need to be strengthen (continuous sampling points) to claim the net effect of liming treatment on N_2O emissions.

4.2 | Unexpected liming effect on soil CO₂ fluxes

Soil respiration is not sensitive to moisture under low temperatures ($<5^{\circ}$ C) but more responsive at high (10° C- 20° C) temperature (Luo & Zhou, 2006). The observed decrease in CO₂ emissions in both treatments as the WFPS increased to saturation is therefore consistent with other studies (e.g., Hofman & Cleemput, 2004; Linn & Doran, 1984), but the liming effect on soil CO₂ fluxes is not.

The second hypothesis of this experiment (that CO₂) emissions will increase following the application of aglime in soil) was formulated according to the premise that carbonate contributed by the aglime will ultimately be released into the atmosphere as CO₂ (IPCC, 2006). The results of this study challenge this hypothesis as CO₂ emissions did not increase in the limed treatment compared with the control. The CO₂ emissions appeared to be even lower in the limed treatment. This is not the first time that the mass balance approach proposed by the IPCC Tier 1 methodology has been challenged. For instance, Biasi et al. (2008) used an isotope marker to differentiate CO₂ derived from lime and biotic respiration and showed that a maximum of 12% of monthly CO2 emissions from cultivated peatland originated from lime, equivalent to one sixth of the lime applied released as CO₂. The decrease of CO₂ emissions in the limed treatment could be the result of stimulating microbial respiration and the production of weak carbonic acid, which in turn reacts with the aglime to form HCO₃⁻ instead of CO_2 (Bramble et al., 2019).

Moreover, apart from the direct release of CO₂ from aglime upon dissolution, aglime and subsequent increases in pH can also affect soil C dynamics by first enhancing the priming effects on SOC decomposition and then release SOC-derived CO₂ (SOC_CO₂) emissions (e.g., Grover et al., 2017). The lower CO₂ emissions in the limed treatment in this study further highlight the negligible influence of changes in soil pH on C mineralisation in this soil, in contrast with recent observations by Aye et al. (2017), which suggested that increases in soil pH by aglime application enhanced native C priming effects through greater microbial biomass and activity. Consequently, the increase in C mineralisation should have increased the SOC_CO2 emissions following the application of aglime (e.g., Dumale et al., 2011). However, the magnitude and dynamics of the C priming effect largely depend on the quality and initial content of C and the resulting nutrient supply to decomposing organisms (Bramble et al., 2021) together with pH dynamics. The potential decrease in SOC mineralisation following aglime application in this study related to lower CO2 emissions could also be due to: (i) increased microbial C-use efficiency, as soil microbial communities use less energy maintaining intracellular pH or there is a change in community composition (Pal et al., 2007), (ii) a more pronounced SOC stabilizing effect and calcium (Ca²⁺) in the soil structure (Muneer & Oades, 1989; Wachendorf, 2015). Indeed, Ca²⁺ is expected to have a greater stabilizing effect on SOC (and aggregate) through its role in the formation of clay-polyvalent cation-organic matter complexes (Clough & Skjemstad, 2000), consequently altering SOC susceptibility to leaching and microbial decomposition. This hypothesis is further demonstrated by the lower leached DOC concentrations in the limed treatment but limited by the lack of soil sampling during the incubation period. In the conditions of this study, the net effect of aglime on the SOC pool, which is hard to determine due to the opposing processes of mineralisation (SOC decrease) and stabilisation by soil structure improvement (SOC increase), tended to be in favour of the second explanation: SOC stabilisation. Further studies are therefore needed to understand the controversial concept of microbial C-use efficiency in the context of varying soil pH, making it necessary to monitor the evolution of SOC during the incubation period.

Finally, and as mentioned above for N_2O emissions, it can be argued that some of the aglime applied to the soil was rapidly leached, suggesting that the decrease in soil pH was fast enough not to cause an increase in C mineralisation and SOC_CO_2 emissions. To obtain a complete picture of the GHG mitigating potential of liming practices, CH_4 emissions should also be monitored in future studies, as Weslien et al. (2009) observed a negative

correlation between soil CH_4 fluxes and pH on a forestry organic soil. Future studies should also aim to create C and N budgets for the entirety of the experiment. This would require continuous CO_2 , CH_4 and N_2O measurements, leachate measurements, and also total and microbial C and N measurements before and after the experiment in line with Ammann et al. (2009).

5 | CONCLUSION

To fulfil the European Commission's objective of reaching a 55% reduction of GHG by 2030, the agricultural sector must have strategies to reduce the emissions of its main GHGs, which include the potent nitrous oxide. Liming is a long-standing agricultural practice that has greatly benefited the productivity of acidic soils. Our findings emphasise the need to consider aglime management practices for mitigating GHG emissions. A significant reduction in gas emissions (N₂O–CO₂) as high as 11.3% was observed for the limed treatment compared with the control in this short-term study, with direct CO₂ emissions from aglime significantly lower than those of the control treatment, emphasizing that further studies are needed to understand the fate of C from carbonate added to soils in different agronomic systems.

AUTHOR CONTRIBUTIONS

Camille Rousset: Conceptualization; investigation; writing – original draft; methodology; visualization; writing – review and editing; software; formal analysis; data curation. Henri Brefort: Conceptualization; investigation; writing – review and editing; methodology. Mustapha Arkoun: Funding acquisition; writing – review and editing; resources. Olivier Mathieu: Methodology; writing – review and editing; resources. Catherine Hénault: Conceptualization; investigation; funding acquisition; writing – review and editing; validation; methodology; project administration; supervision; resources; data curation.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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