The Effects of pH Change through Liming on Soil N2O Emissions

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Article

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Abstract: Nitrous oxide (N$_2$O) is an overwhelming greenhouse gas and agricultural soils, particularly acidic soils, are the main source of its release to the atmosphere. To ameliorate acidic soil condition, liming materials are added as an amendment. However, the impact of liming materials has not been well addressed in terms of exploring the effect of soil pH change on N$_2$O emissions. In the present study, a soil with pH 5.35 was amended with liming materials (CaMg(CO$_3$)$_2$, CaCO$_3$, Ca(OH)$_2$ and CaO) to investigate their effects on N$_2$O emissions. The results indicate that application of liming materials reduced the magnitudes of N$_2$O emissions. The maximum reduction of soil N$_2$O emissions took place for Ca(OH)$_2$ treatment when compared to the other liming materials, and was related to increasing soil pH. Mineral N, dissolved organic C, and microbial biomass C were also influenced by liming materials, but the trend was inconsistent to the soil pH change. The results suggest that N$_2$O emission mitigation is more dependent on soil pH than C and N dynamics when comparing the different liming materials. Moreover, ameliorating soil acidity is a promising option to mitigate N$_2$O emissions from acidic soils.

Keywords: lime; mineral nitrogen; soil pH; organic carbon; microbial biomass; N$_2$O

1. Introduction

Soil acidity is a master variable that hinders plant growth by limiting nutrient availability and thus impacts both the quantity and quality of crops. Soil acidification occurs very slowly naturally as soil is weathered, but this process is accelerated by intensive agriculture [1,2]. Soil acidity is expressed in terms of pH, and its extent and degree impact a wide range of soil biogeochemical properties. Soil acidity also has marked effects on soil microbial communities and their pertinent processes.

Soil acidification is a natural and very slow process that takes over hundreds of years to develop. However, it may reach its greatest expression within a few years under intensive agricultural practices and in humid regions where rainfall is sufficient to leach down the nutrients [3]. Thus, although most processes developing soil acidification are natural, anthropogenic activities have a major impact on some of them. In fact, several reasons may contribute to soil acidification and excessive use of nitrogen (N) is one of them [3].

To obtain high crop production in intensive crop-growing areas, excessive application of N fertilizers has been carried out for years, but when it is excessive, it leads to soil acidification [4]. According to estimations [3], the application of nitrogen fertilizer in arable lands of China usually ranges from 200 to 500 kg N ha^{-1} yr^{-1}. Aside from the beneficial effects of high N fertilizer application, devastating impacts and environmental risks have also been observed including eutrophication, nitrous oxide (N_2O) emissions, and soil acidity [5,6]. Researchers have demonstrated that nitrate and ammonium applied to soils can generate 20 to 33 kmol hydrogen ions (H$^+$) ha^{-1} yr^{-1} under exhaustive growing systems [3]. This indicates that the application of N can drive soil acidification.

Soil acidity can be offset with alkaline materials that provide conjugate bases such as CO_3^{2-} and OH$^-$ of weak acids. These anionic bases react with H$^+$ and form weak acids. For example:

$$CO_3^{2-} + 2H^+ \rightarrow H_2CO_3 \tag{1}$$

Generally, liming materials are applied in the forms of hydroxides or oxides containing magnesium (Mg) or calcium (Ca), which form hydroxide ions in water.

$$CaO + H_2O \rightarrow Ca(OH)_2 \rightarrow Ca^{2+} + 2OH^- \tag{2}$$
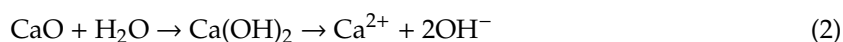
Most liming materials, whether they are carbonates, hydroxides, or oxides, react with CO_2 and H_2O to generate bicarbonates (HCO$_3^-$) when added to acidic soils. As a result, partial pressure of CO_2 in the soil is high enough to proceed such reactions forward, for instance:

$$CaMg(CO_3)_2 + 2H_2O + 2CO_2 \rightleftharpoons Ca^{2+} + Mg^{2+} + 4HCO_3^- \tag{3}$$
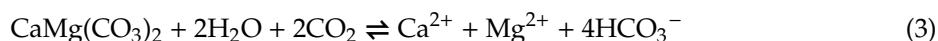
The resultant bicarbonates, Ca and Mg, counteract the acidity.

Liming acidic soils not only raises soil pH, but also alters biochemical processes and nutrient cycling. The rise in soil pH following lime application substantially triggers the N transformation processes [7], markedly controls the microbial processes of nitrification and denitrification, and thus influences N_2O production and emission. However, the subsequent effects of lime application on N_2O emissions is ambiguous and contrary hypotheses have been proposed by the scientific community. For instance, a laboratory incubation study proposed that increasing soil pH may substantially decrease emissions of N_2O from acidic agricultural soils [8]. In contrast, some scientists have reported that lime application and subsequent rise in soil pH caused increased soil N_2O emissions from arable acidic soils [9,10].

Keeping in mind the importance of liming acidic soils, we hypothesized that the application of lime materials can trigger N transformations following soil pH change and subsequently influence N_2O emissions in a way that would be interesting to further elucidate. Thus, the current study was conducted with the aim to examine and shed further light on the pH change effects of various liming materials on N_2O emissions from acidic agricultural soils.

2. Materials and Methods

2.1. Soil and Liming Materials

Soil was obtained from a rapeseed-rice cropping system, located in Xianing (a city of central China; 29°88′209″ N, 114°39′416″ E). According to Soil Survey staff [11], the soil is classified as Ultisol.

Soil (0–20 cm) was sampled from the selected field after rice harvest from multiple-points. A composite soil sample was made by mixing subsamples. Plant residues (straw and roots) were separated from soil. After shifting in the laboratory, soil was dried in the open air, crumbled, and then sieved through a 2 mm sieve. The basic soil chemical and physical analysis [12] was performed prior to onset of the experiment. Soil texture was silty clay loam. The main characteristics of soil are given in Table 1. Different liming materials (dolomite $CaMg(CO_3)_2$, calcium hydroxide $(Ca(OH)_2$, calcium carbonate $CaCO_3$, and calcium oxide CaO) used in the present study were purchased from Xinjing Chemicals Co. Ltd. (Xiaogan, Hubei, China).

Table 1. Some selected physical and chemical characteristics of the tested soil.

$pH_{(H2O)}$	Total C (g/kg)	Total N (g/kg)	NO_3^--N (mg/kg)	NH_4^+-N (mg/kg)	Bulk Density (g/cm^3)	Cation Exchange Capacity (cmol$_c$/kg)
5.35	11.5	1.2	15.6	46.9	1.39	10.8

2.2. Experimental Setup for Collection and Analysis of N_2O

Initially, air dried soil without any amendment was incubated in a plastic (polyethylene terephthalate) tub with 20% gravimetric water content (60% water filled pore space) at a temperature of 25 ± 1 °C for one week (Figure 1). After one week of initial incubation, incubated wet soil (100 g on dry basis) from the tub was placed in glass jars. Liming materials were added separately to the soil. The application dose of each liming material was 1 g kg^{-1} dry soil with a particle size ≤0.3 mm. Treatments for the present study were as follows: (i) $Ca(OH)_2$, (ii) CaO, (iii) $CaCO_3$, (iv) $CaMg(CO_3)_2$, and (v) control (soil without any amendment). Each treatment had three replicates. Treated soils in jars were placed in an electric-automated chamber (S-400-HP) and incubated at 25 ± 0.5 °C in the dark for four weeks (28 days). During incubation, a plastic sheet with pin holes (about 30) was used on the top of each jar to reduce water loss, but permit gas exchange. Soil water content in each jar was sustained at 20% throughout the study by weighing jars and refilling with distilled water on a daily basis.
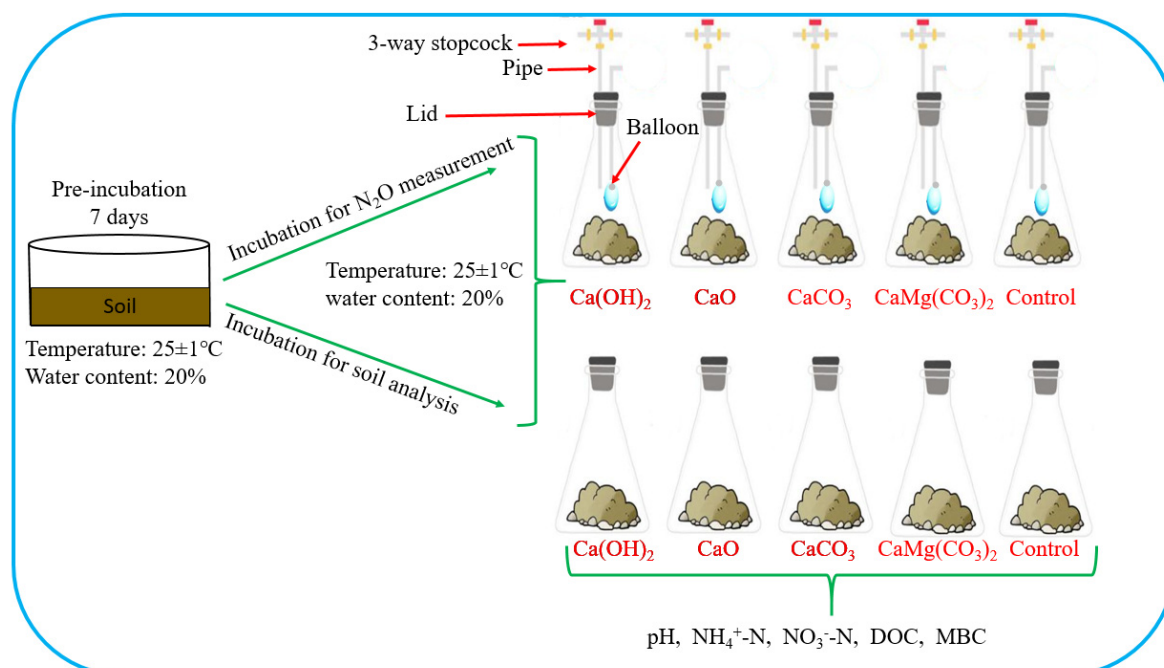
Figure 1. Schematic diagram of the experimental setup.

Gas from the headspaces of jars, equipped with air-tight lids holding a 3-mm diameter pipe, was collected at days 1, 3, 5, 7, 10, 13, 16, 20, 24, and 28 using a special air-tight syringe made for sampling purposes. On gas sampling day, the tops of jars were uncovered prior to gas sampling and soil in the jars was allowed to be exposed to ambient air for 30 to 40 min. After that, the jars were closed with air-tight lids and gas samples were taken immediately after closure to know the initial concentration of gas in the jars. Another gas sample from headspace was collected after 60 min to know the change in gas production. The gas samples were analyzed for N_2O concentration using a gas chromatograph system (7890A, Agilent technology, Santa Clara, CA, USA). The concentration of N_2O in the gas sample was calculated using the equation as given below [13].

$$F = \rho \times V/W \times \Delta c/\Delta t \times 273/(T + 273) \tag{4}$$

In Equation (1), F denotes the rate of N_2O–N emission ($\mu g \, kg^{-1} \, h^{-1}$); ρ denotes the density ($kg \, m^{-3}$) of N_2O gas; V denotes the volume (m^3) of headspace of jars; W denotes soil weight (kg); Δc denotes change in gas concentration during closure time of jars; Δt denotes the time period of closure (h) of the treatment jars; and T denotes the temperature at which the experiment was conducted (25 °C).

The cumulative emissions of N_2O ($\mu g \, kg^{-1}$) for the whole period of study were calculated based on the following formula [14].

$$\text{Cumulative N2O emission} = \sum_{i=1}^{n} (Ri \times 24 \times Di) \tag{5}$$

where *Ri* is the N_2O emission rate ($\mu g \, kg^{-1} \, h^{-1}$); *Di* are days between the sampling periods; and *n* is the number of samples.

2.3. Experimental Setup for Soil Analysis

A separate experiment to that for gas analysis was concurrently performed for soil analysis. Treatments, pre-incubation period, temperature, and moisture conditions for the soil analysis study were identical as that for the gas analysis setup. After pre-incubation, a weight of 200 g soil was incubated after being placed in 1000 mL beakers. Soil sub-samples from jars were taken after one day of imposing treatments and then on a weekly basis over 28 days.

For pH determination of the soil-sub samples, a soil slurry was made by performing a 1:2.5 ratio suspension of soil:distilled water [12]. The slurry was shaken in an orbital shaker for 40 min, and the pH was tested using a pH-meter (2FPHS, Wincoms Co. Ltd., Shanghai, China) after 30 min of shaking. Soil was subjected to specific extraction for the subsequent determination of the mineral contents of soil nitrogen (NO_3^-–N and NH_4^+–N) by adding 1 M KCl (5 mL for 1 g soil), shaking for 60 min, and subsequently using a flow injector system analyzer (SEAL Co. Ltd., Henstedt-Ulzburg, Germany) [15]. Chloroform fumigation specific extraction method was adopted for testing microbial biomass C [16]. Dissolved organic C content in soil was determined by extracting the soil with distilled water (1:5, soil:distilled water) and using Elementar system analysis (Vario, Elementar-CN, Hanau, Germany).

2.4. Data Analysis

Data pertinent to soil and gas parameters were analyzed using Analysis of Variance (ANOVA) one-way analysis of variance. Tukey's test was employed to identify significant differences for treatments of their mean results. The Kolmogorov–Smirnov test for the normality distribution of variables was performed before proceeding further for ANOVA [17]. All data were statistically evaluated using Windows-based software Statistical Package for the Social Sciences (SPSS) Statistics 23.

3. Results

3.1. Soil pH

Soil pH was statistically significantly ($p \leq 0.01$) different among the treatments of liming materials. Soil pH before the immediate day of adding liming materials was 5.35, and liming of soil rapidly increased pH (Figure 2). On day 1, soil pH in all treatments was substantially higher than that of the control and thereafter continued to gradually increase up until the end of the study. The highest value of soil pH corresponded to $Ca(OH)_2$ treatment on day 28. The pH values were 7.21, 6.99, 6.70, 6.43, and 5.30 for $Ca(OH)_2$, CaO, $CaCO_3$, $CaMg(CO_3)_2$, and the control, respectively, on day 28 of the experiment.
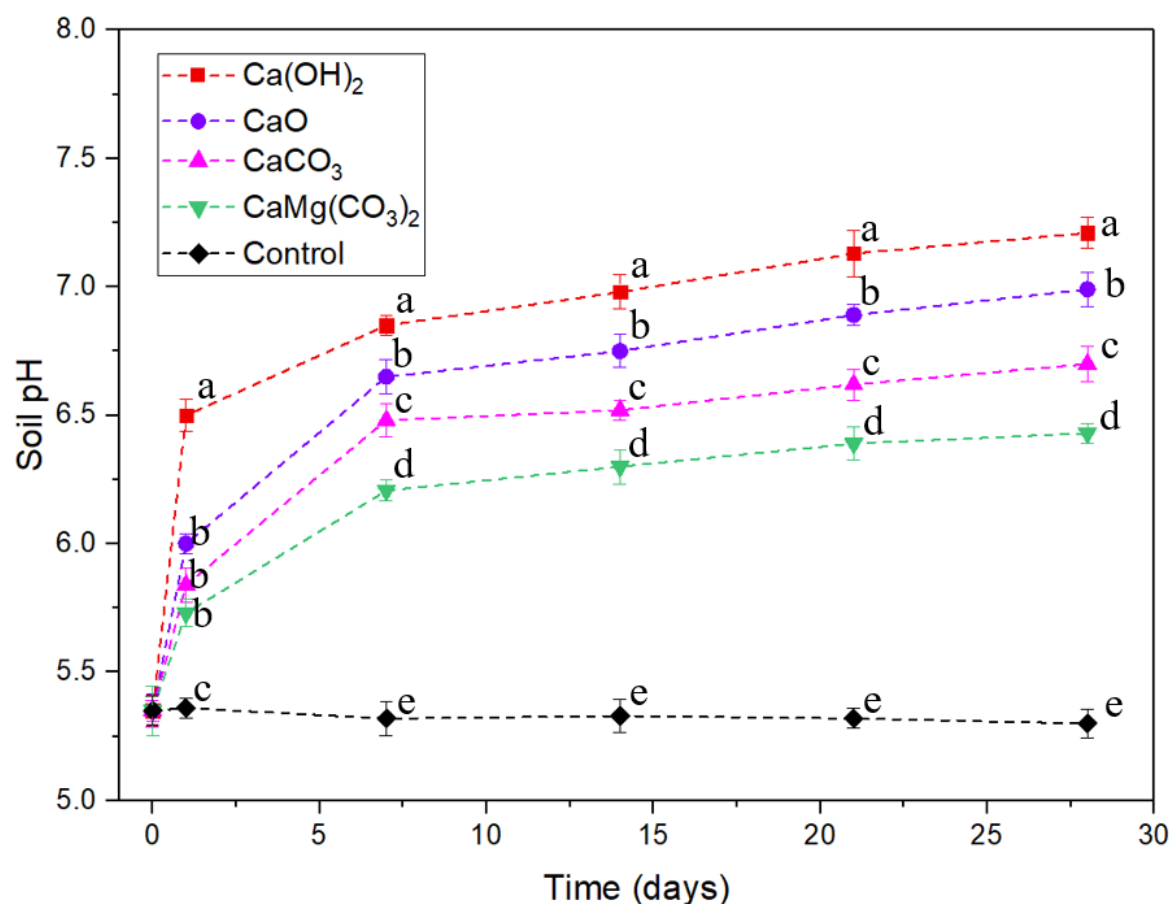
Figure 2. Dynamics of soil pH following the application of liming materials. Vertical bars denote the error bars of the mean of three replicates. Values at the same time followed by different letters indicate significant differences between different treatments ($p < 0.05$).

3.2. Soil Mineral–N (NH_4^+–N and NO_3^-–N)

Soil NH_4^+–N concentrations were highly and significantly ($p \leq 0.01$) influenced by the addition of liming materials. NH_4^+–N concentration before the immediate day of adding liming materials was 35 mg kg^{-1}, whereas the addition of liming materials caused diverse patterns of NH_4^+–N concentrations (Figure 3). The $CaCO_3$, $CaMg(CO_3)_2$ and control treatments showed continuous decline of NH_4^+–N concentrations throughout the study period. However, NH_4^+–N concentration in the $Ca(OH)_2$ and CaO treatments declined on day 1 of the onset of the study, increased on day 2, and afterward speedily decreased throughout until the end of the experiment. The NH_4^+–N concentrations were 9.1, 10.9, 6.8, 12.0, and 20.2 mg kg^{-1} in the $Ca(OH)_2$, CaO, $CaCO_3$, $CaMg(CO_3)_2$ and control treatments, respectively, on day 28 of the study.
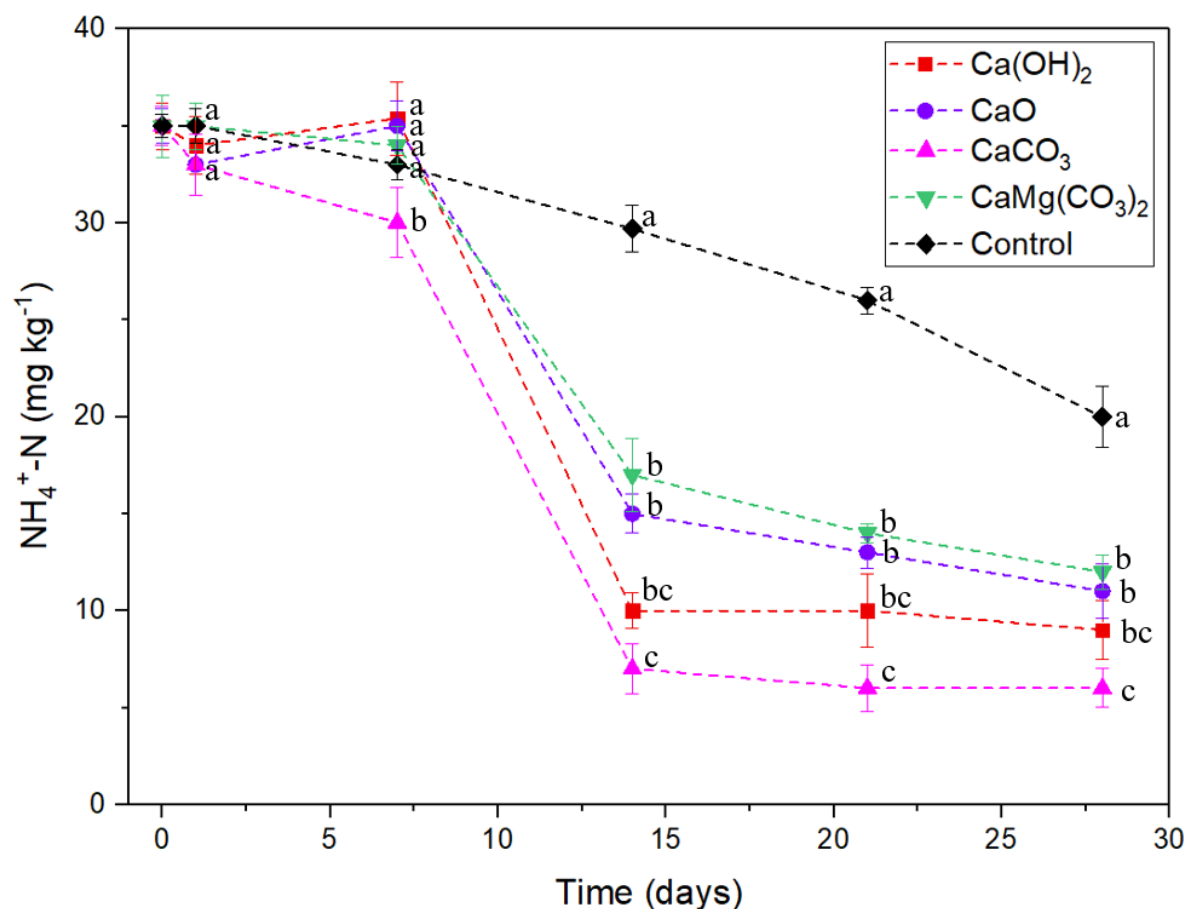
Figure 3. Variations in soil ammonium concentrations following the application of liming materials. Vertical bars denote the error bars of the mean of three replicates. Values at the same time followed by the different letters indicate significant differences between different treatments ($p < 0.05$).

The amendment of liming materials significantly ($p \leq 0.01$) augmented soil $NO_3^- $–N concentrations (Figure 4). The trend of increase of NO_3^-–N concentration kept continuing in all treatments until the end of the study. The maximum concentration of NO_3^-–N was observed in the $CaCO_3$ treatment on day 28 of the study. The NO_3^-–N concentrations were 60.2, 52.4, 75.1, 50.0, and 43.9 mg kg^{-1} in the $Ca(OH)_2$, CaO, $CaCO_3$, $CaMg(CO_3)_2$, and control, respectively, on day 28 of the study.
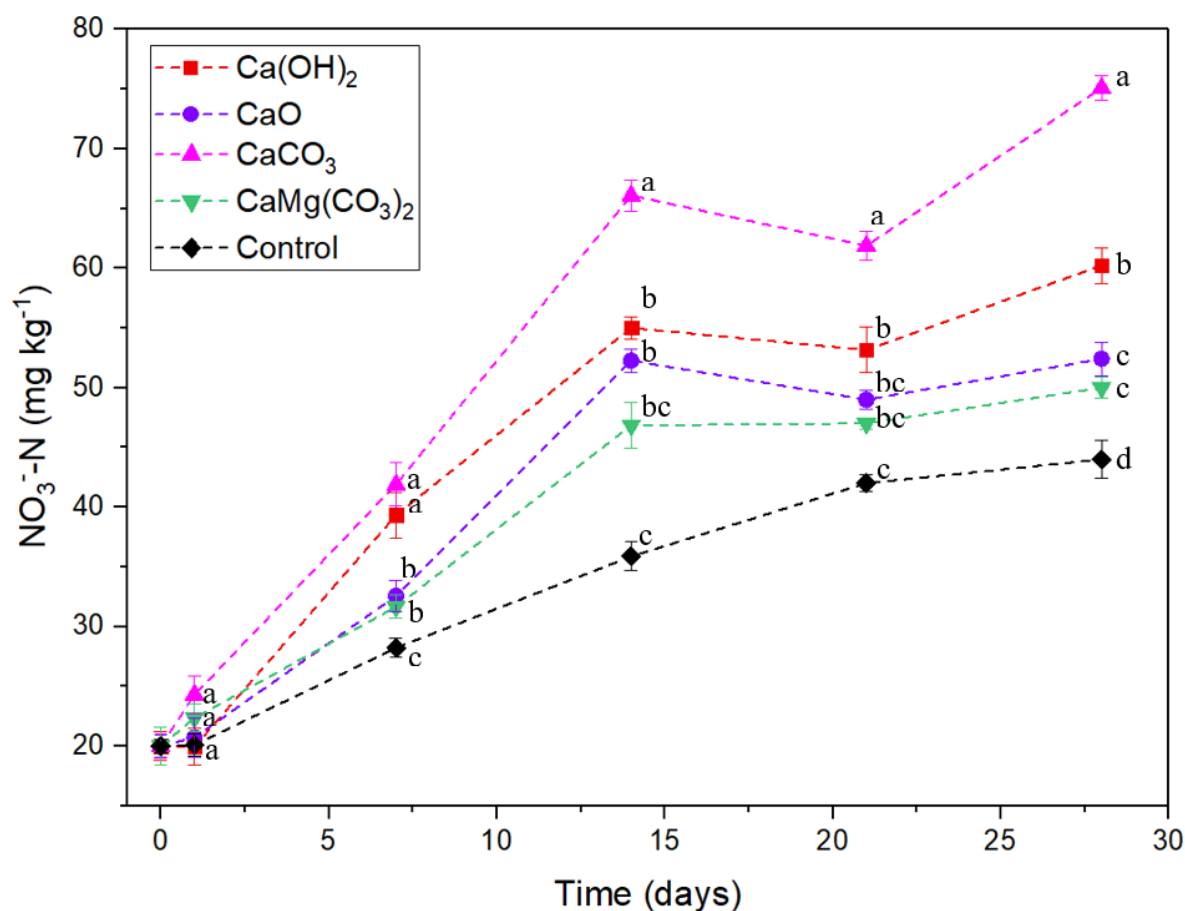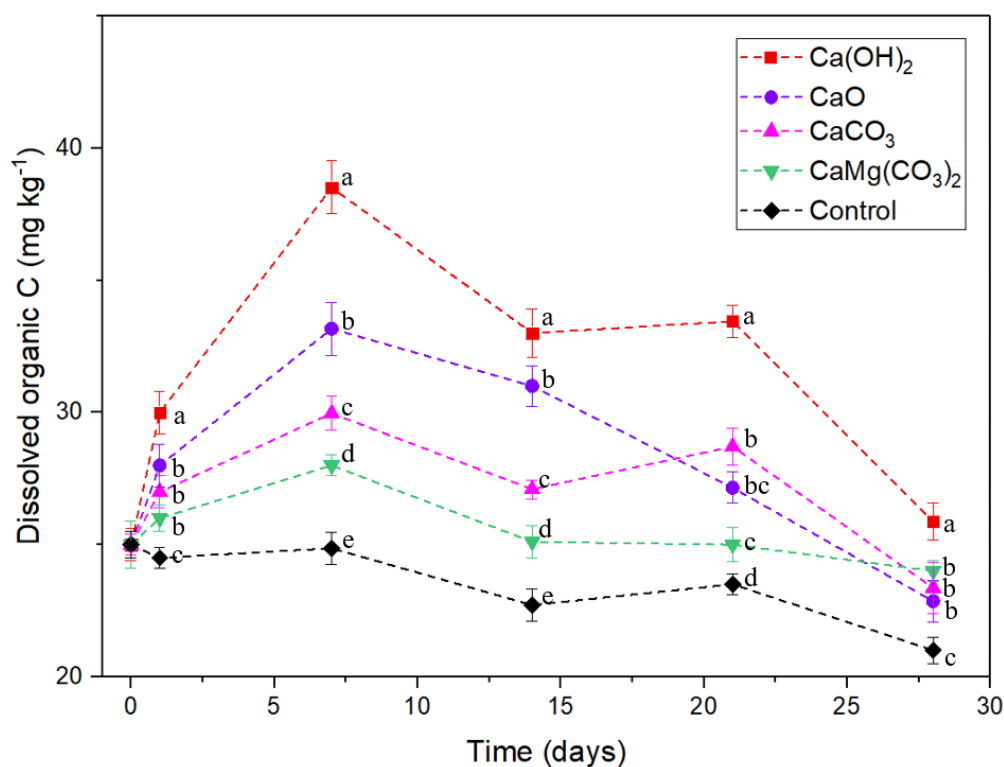
Figure 4. Variations in soil nitrate concentrations following the application of liming materials. Vertical bars denote the error bars of the means of three replicates. Values at the same time followed by different letters indicate significant differences between different treatments ($p < 0.05$).

3.3. Dissolved Organic C and Microbial Biomass C

Addition of liming materials significantly ($p \leq 0.01$) impacted the microbial biomass C (MBC) as well as dissolved organic C (DOC) in soil. Before the addition of liming materials, the DOC content was 25 mg kg^{-1} and instantly increased on day 1 in all treatments, except for the control (Figure 5). The DOC contents reached maximum values of 38.5, 33.2, 30.1, 28, 24.9 mg kg^{-1} on day 7 in the Ca(OH)$_2$, CaO, CaCO$_3$, CaMg(CO$_3$)$_2$, and control treatments, respectively, and afterward declined until the end of the study.

In the case of MBC contents, all the liming treatments showed an increment on day 1, while a divergent trend was observed afterward (Figure 6). Only Ca(OH)$_2$ treatment showed a rise in MBC content after day 1, reached the maximum at 59 mg kg^{-1} on day 14, and after that gradually declined and reached 49.1 mg kg^{-1} at the end of the experiment, whereas MBC contents decreased in all other treatments of CaO, CaCO$_3$, and CaMg(CO$_3$)$_2$ as well as the control over the entire study period.

Figure 5. Fluctuations in soil dissolved organic carbon following the application of liming materials. Vertical bars denote error bars of mean of three replicates. Values at the same time followed by different letters indicate significant differences between different treatments ($p < 0.05$).
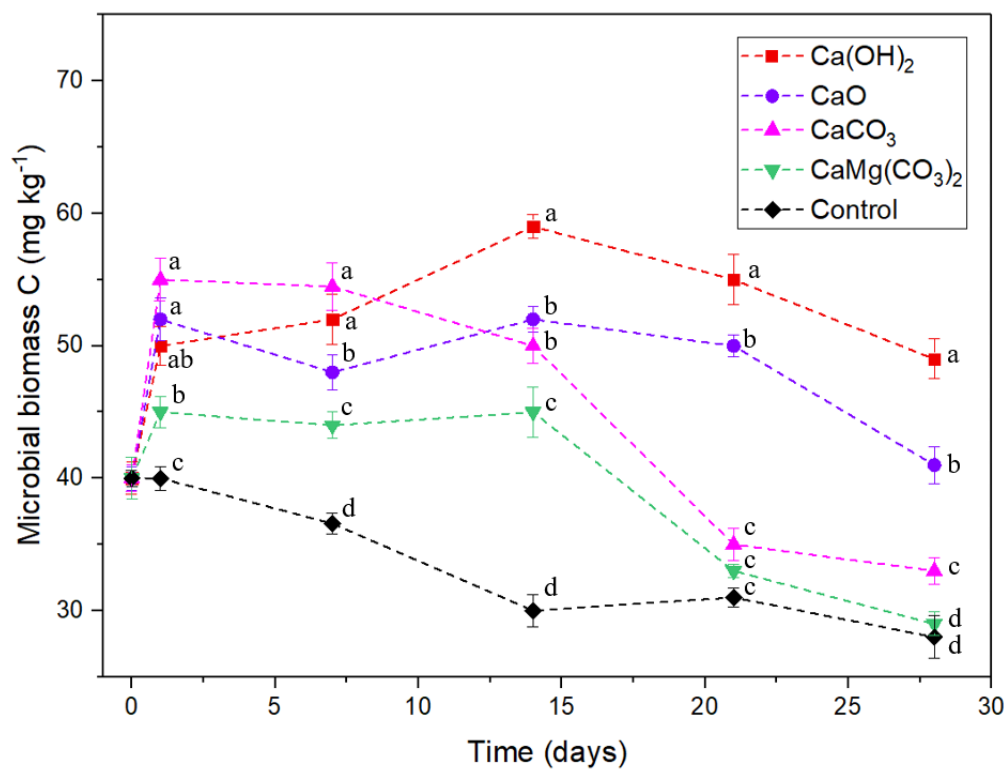
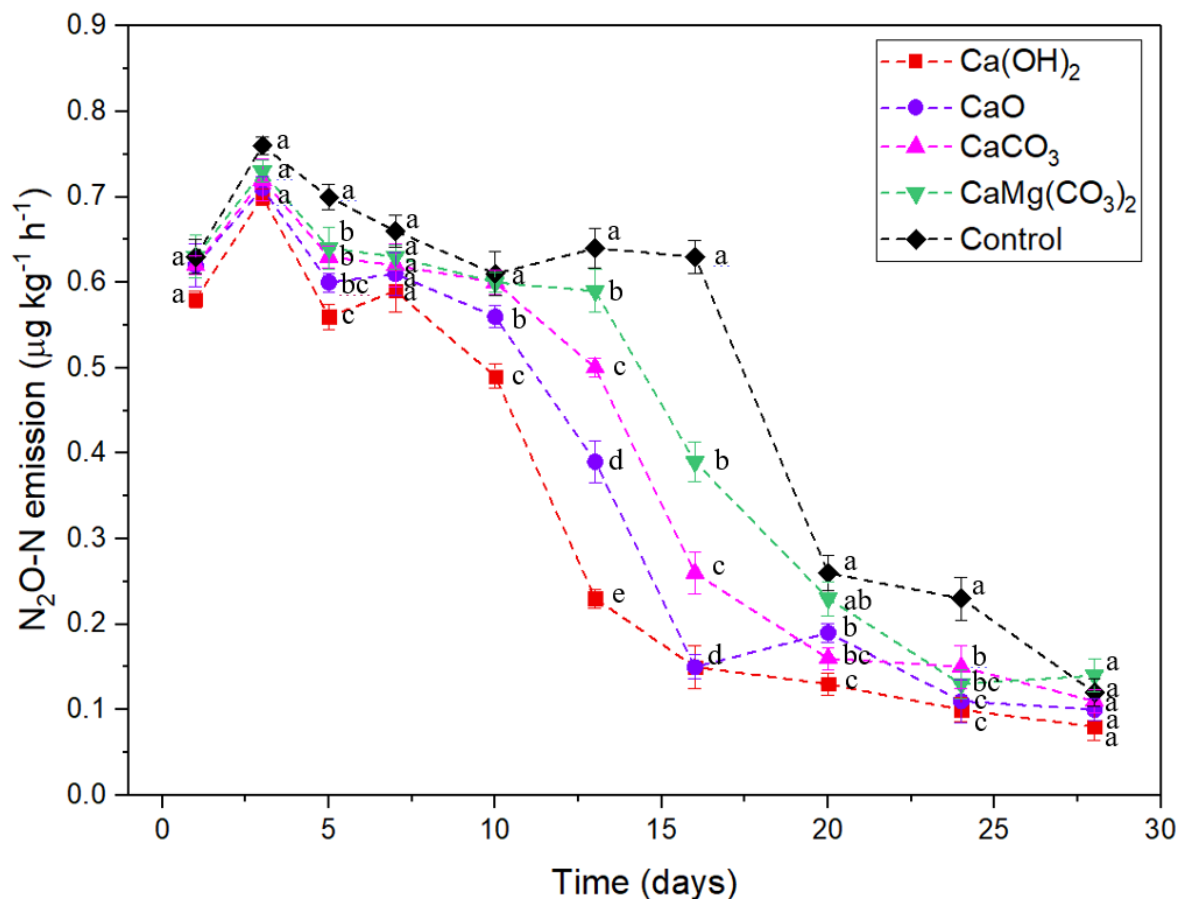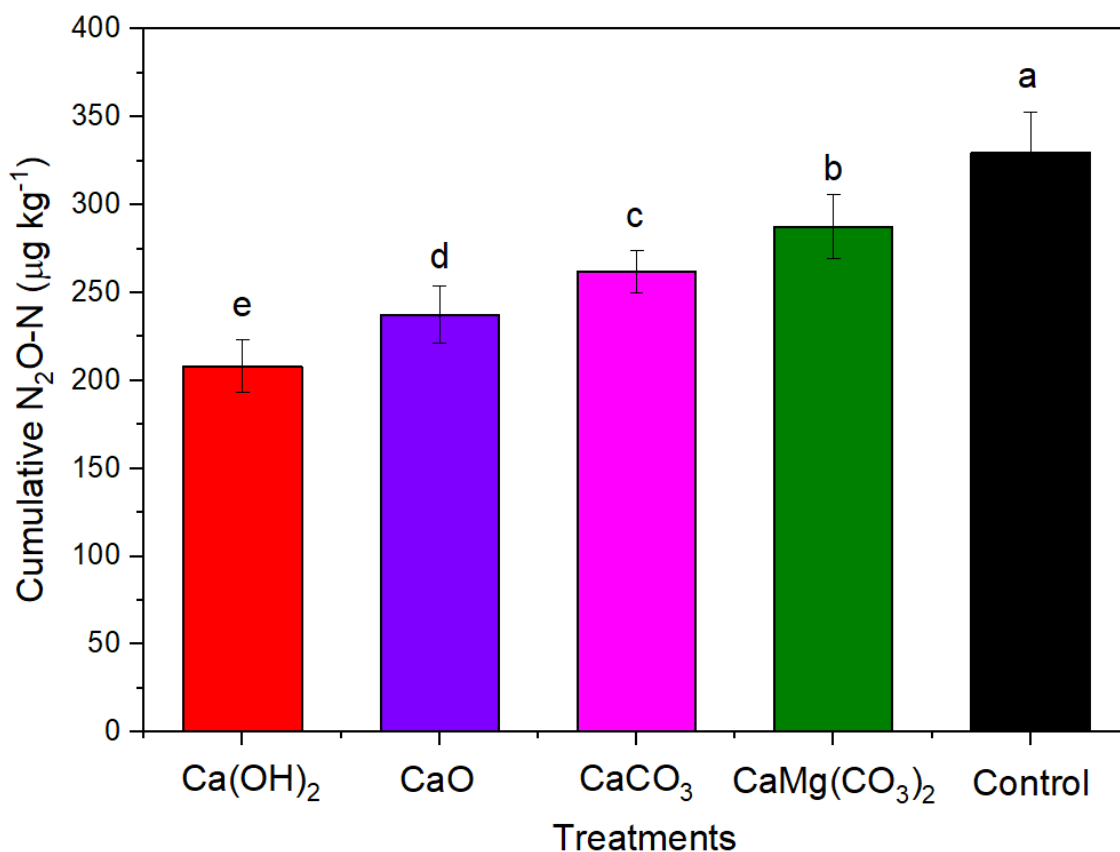
Figure 6. Fluctuations in soil microbial biomass carbon following the application of liming materials. Vertical bars denote the error bars of the means of three replicates. Values at the same time followed by different letters indicate significant differences between different treatments ($p < 0.05$).

3.4. Nitrous Oxide (N_2O) Emissions

Nitrous oxide emissions were significantly ($p \leq 0.01$) affected by the application of liming materials. The N_2O emission rate and the cumulative soil N_2O emissions (329.52 µg kg^{-1}) were highest in the control among all the treatments (Figures 7 and 8). The N_2O emissions increased on day 1 following the addition of liming materials, and then started to decline, with variant magnitudes (Figure 7). The decrease in N_2O emissions was sharper in $Ca(OH)_2$ than that of the other liming treatments, and indeed in the control. The lowest emission rate and cumulative N_2O emissions were observed in the $Ca(OH)_2$ treatment. The cumulative N_2O emissions in the $Ca(OH)_2$, CaO, $CaCO_3$, $CaMg(CO_3)_2$, and control treatments were 208.08, 237.60, 261.72, 287.64, and 329.52 µg kg^{-1}, respectively (Figure 8).

Figure 7. Soil N_2O emissions following the application of liming materials. Vertical bars denote the error bars of the means of three replicates. Values at the same time followed by different letters indicate significant differences between different treatments ($p < 0.05$).

Figure 8. Cumulative soil N_2O emissions following the application of liming materials. Vertical bars denote the error bars of the means of three replicates. Different letters (from *a* to *e*) denote significant differences ($p \leq 0.05$) among the means of treatments.

4. Discussion

Acidic soils are generally considered as less efficient for high crop production. To ameliorate acidic soils, farmers usually amend them with liming materials. Application of liming materials has dual benefits of raising soil pH as well as supply essential elements, mainly Ca and Mg. In the present study, the liming materials used were $Ca(OH)_2$, CaO, $CaCO_3$, and $CaMg(CO_3)_2$. Application of all these liming materials obviously influenced N_2O emissions, but the magnitudes of the N_2O emissions dramatically altered with soil pH. In fact, high N_2O emissions were observed at low pH levels (without lime application) in the acidic soil in the present study.

High magnitudes of N_2O emissions from low pH soils can be explained by incomplete denitrification and less activity or complete inhibition of N_2O–reductase. Nitrous oxide reductase (N_2O–R) is the sole enzyme of the denitrification process, which reduces N_2O to N_2 at neutral, near neutral, or above 7 pH [18]. Therefore, higher magnitudes of N_2O emissions are expected from soils at low pH relative to higher pH values because of the incomplete denitrification process [19,20]. In the present study, N_2O emissions were perceptibly mitigated by the application of all selected liming materials. However, the highest decline in cumulative as well as emission rates of N_2O occurred in the $Ca(OH)_2$ treatment, and this was possible due to the highest pH value. Kunhikrishnan et al. [21] also indicated that the pH value could prominently control N_2O production and emissions, and Bakken et al. [22] commented that the possible mechanism involved in low magnitudes of N_2O emissions in limed soils at high pH values was pertinent to the activities of N_2O–reductase. It has been shown that the application of liming materials improved the activities of N_2O–reductase for N_2O reduction [23], and magnitudes of soil N_2O emissions are unswervingly controlled by pH [8,24]. These studies demonstrated that N_2O–reductase was functional at higher pH

relative to low pH, which led to a complete denitrification process and low N_2O emissions at high pH levels.

Results of raising soil pH regarding the effect on N_2O emissions have been proposed by several researchers. Stevens and Laughlin [25] reported that raising the soil pH from 6.5 to 8 eminently reduced N_2O emissions. Qu et al. [26] reported that acidic soils produced higher magnitudes of N_2O emissions, whereas neutral pH soils showed less magnitudes of N_2O emissions. Khan et al. [27] found that the application of calcium hydroxide to soil at the dose of 5.63 g kg^{-1} soil significantly decreased N_2O emissions by increasing soil pH from 5.2 to 7.6. Additionally, an 80-day laboratory study revealed that $Ca(OH)_2$ amendment (1.1 to 5.6 g kg^{-1} soil) substantially reduced N_2O emission [28]. Moreover, some other experiments showed the following: application of $Ca(OH)_2$ at the dose of 7.3 g kg^{-1} soil mitigated cumulative emissions of N_2O from 547 g ha^{-1} to 46 g ha^{-1} in a soil with a pH of 4.71 [29]. A 2-year research showed that increasing the pH from 4 to 5.5 by $CaCO_3$ application dwindled N_2O emissions from 0.96 mg m^{-1} d^{-1} to 0.88 mg m^{-1} d^{-1} [30]. The mitigation of N_2O emissions from limed soils showed that pH plays an imperative role in regulating such N_2O release to the atmosphere [31].

In the present study, the addition of liming materials greatly impacted mineral N concentrations displaying a quick decline of NH_4^+–N with time, indicating that the nitrification process sped up, as linked to the concurrent rise of NO_3^-–N concentrations. Higher NO_3^-–N concentrations at relatively higher soil pH levels advocate that microbes consumed N_2O as an electron acceptor instead of NO_3^-–N. It can be observed from these results that complete denitrification occurred, rendering N_2O to N_2 conversion in all liming material treated soils, and thus correspondingly, low magnitudes of N_2O emissions occurred. Moreover, it is interesting to report herein that the trend and behavior of N_2O release from liming material amended soils corresponded with the changes in NH_4^+–N and NO_3^-–N concentrations, but the degree of the mitigation of N_2O emissions did not follow the same pattern. The most rapid changes of mineral N dynamics were observed in the $CaCO_3$ treatment, whereas the highest reduction of N_2O emissions occurred in the $Ca(OH)_2$ treatment. The discrepancies between the degrees of N_2O emission magnitudes following liming material application is plausible because of the potential of soil pH manipulation.

In addition to mineral N dynamics, the application of all liming materials influenced dissolved organic C, which is believed to be a readily available C substrate for microbial growth prolongation and proliferation, leading to processing nitrification and denitrification producing N_2O [32]. It is interesting to note that the changes in MBC comparing the end values with the starting values: ca. −10 mg/kg soil for the control versus are ca. +10 mg/kg for treatment $Ca(OH)_2$. Dissolved organic C acted as a substrate for microbes, conjecturing that available C favored N_2O reduction. Furthermore, high contents of MBC in the liming material added soils were detected when compared to the control, which indicated the likely high reduction of N_2O emissions.

5. Conclusions

The present research showed that the application of liming materials reduced magnitudes of N_2O emissions. The pronounced and maximum reduction of soil N_2O emissions occurred in the $Ca(OH)_2$ treatment through increasing soil pH when compared to the other liming materials tested. The results suggest that N_2O emission mitigation is more dependent on soil pH than on C and N dynamics when capering different liming materials. Moreover, ameliorating the soil acidity condition is a promising option to alleviate N_2O emissions from acidic soils. The results can be considered of environmental relevance, and further research in this regard could be interesting, especially in the current context of global warming due to a variety of greenhouse gases released to the atmosphere from different compartments and due to various anthropogenic activities.

Author Contributions: Conceptualization, M.S. and R.H.; Formal analysis, M.S., Y.W., L.W., R.H. and S.L.; Investigation, M.S.; Methodology, M.S., Y.W., L.W., P.X., Z.S., X.X. and Y.J.; Software, M.S. and A.Y.; Writing—original draft, M.S.; Writing—review & editing, M.S., A.Y., A.N.-D. and S.L. All authors have read and agreed to the published version of the manuscript.

References

1. von Uexküll, H.R.; Mutert, E. Global extent, development and economic impact of acid soils. *Plant. Soil* **1995**, *171*, 1–15. [CrossRef]

2. Goulding, K. Soil acidification and the importance of liming agricultural soils with particular reference to the United Kingdom. *Soil Use Manag.* **2016**, *32*, 390–399. [CrossRef] [PubMed]

3. Guo, J.; Liu, X.; Zhang, Y.; Shen, J.; Han, W.; Zhang, W.; Christie, P.; Goulding, K.; Vitousek, P.; Zhang, F. Significant acidification in major Chinese croplands. *Science* **2010**, *327*, 1008–1010. [CrossRef] [PubMed]

4. 4 Shaaban, M.; Peng, Q.; Lin, S.; Wu, Y.; Zhao, J.; Hu, R. Nitrous oxide emission from two acidic soils as affected by dolomite application. *Soil Res.* **2014**, *52*, 841–848. [CrossRef]

5. Smith, V.H.; Tilman, G.D.; Nekola, J.C. Eutrophication: Impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environ. Pollut.* **1999**, *100*, 179–196. [CrossRef]

6. Erisman, J.W.; Galloway, J.N.; Seitzinger, S.; Bleeker, A.; Dise, N.B.; Petrescu, A.R.; Leach, A.M.; de Vries, W. Consequences of human modification of the global nitrogen cycle. *Philos. Trans. R. Soc. B Biol. Sci.* **2013**, *368*, 20130116. [CrossRef]

7. Shaaban, M.; Peng, Q.; Hu, R.; Lin, S.; Wu, Y.; Ullah, B.; Zhao, J.; Liu, S.; Li, Y. Dissolved organic carbon and nitrogen mineralization strongly affect CO_2 emissions following lime application to acidic soil. *J. Chem. Soc. Pak.* **2014**, *36*, 875–879.

8. Shaaban, M.; Hu, R.; Wu, Y.; Younas, A.; Xu, X.; Sun, Z.; Jiang, Y.; Lin, S. Mitigation of N_2O emissions from urine treated acidic soils by liming. *Environ. Pollut.* **2019**, *255*, 113237. [CrossRef]

9. Nägele, W.; Conrad, R. Influence of pH on the release of NO and N_2O from fertilized and unfertilized soil. *Biol. Fertil. Soils* **1990**, *10*, 139–144.

10. Feng, K.; Yan, F.; Hütsch, B.W.; Schubert, S. Nitrous oxide emission as affected by liming an acidic mineral soil used for arable agriculture. *Nutr. Cycl. Agroecosyst.* **2003**, *67*, 283–292. [CrossRef]

11. Soil Survey Staff. *Keys to Soil Taxonomy*, 11th ed.; USDA Natural Resources Conservation Service: Washington, DC, USA, 2010.

12. Tan, K.H. *Soil Sampling, Preparation, and Analysis*; Marcel Dekker: New York, NY, USA, 1996.

13. Hu, R.; Hatano, R.; Kusa, K.; Sawamoto, T. Soil respiration and net ecosystem production in an onion field in central Hokkaido, Japan. *Soil Sci. Plant Nutr.* **2004**, *50*, 27–33. [CrossRef]

14. Jin, T.; Shimizu, M.; Marutani, S.; Desyatkin, A.R.; Iizuka, N.; Hata, H.; Hatano, R. Effect of chemical fertilizer and manure application on N_2O emission from reed canary grassland in Hokkaido, Japan. *Soil Sci. Plant Nutr.* **2010**, *56*, 53–65. [CrossRef]

15. Kachurina, O.; Zhang, H.; Raun, W.; Krenzer, E. Simultaneous determination of soil aluminum, ammonium-and nitrate-nitrogen using 1 M potassium chloride extraction. *Commun. Soil Sci. Plant. Anal.* **2000**, *31*, 893–903. [CrossRef]

16. Vance, E.D.; Brooks, P.C.; Jenkinson, D.S. An extraction method for measuring soil microbial biomass. *Soil Biol. Biochem.* **1987**, *19*, 703–707. [CrossRef]

17. Razali, N.M.; Wah, Y.B. Power comparisons of Shapiro–Wilk, Kolmogorov–Smirnov, Lilliefors and Anderson–Darling tests. *J. Stat. Model. Anal.* **2011**, *2*, 21–33.

18. Flessa, H.; Wild, U.; Klemisch, M.; Pfadenhauer, J. Nitrous oxide and methane fluxes from organic soils under agriculture. *Eur. J. Soil Sci.* **1998**, *49*, 327–335.

19. Šimek, M.; Cooper, J. The influence of soil pH on denitrification: Progress towards the understanding of this interaction over the last 50 years. *Eur. J. Soil Sci.* **2002**, *53*, 345–354. [CrossRef]

20. Shaaban, M.; Wu, Y.; Peng, Q.-a.; Lin, S.; Mo, Y.; Wu, L.; Hu, R.; Zhou, W. Effects of dicyandiamide and dolomite application on N_2O emission from an acidic soil. *Environ. Sci. Pollut. Res.* **2016**, *23*, 6334–6342. [CrossRef] [PubMed]

21. Kunhikrishnan, A.; Thangarajan, R.; Bolan, N.S.; Xu, Y.; Mandal, S.; Gleeson, D.B.; Seshadri, B.; Zaman, M.; Barton, L.; Tang, C.; et al. Functional relationships of soil acidification, liming, and greenhouse gas flux. *Adv. Agron.* **2016**, *139*, 1–71.

22. Bakken, L.R.; Bergaust, L.; Liu, B.; Frostegard, A. Regulation of denitrification at the cellular level: A clue to the understanding of N_2O emissions from soils. *Philos. Trans. R. Soc. B-Biol. Sci.* **2012**, *367*, 1226–1234. [CrossRef]

23. Samad, M.S.; Bakken, L.R.; Nadeem, S.; Clough, T.J.; de Klein, C.A.M.; Richards, K.G.; Lanigan, G.J.; Morales, S.E. High-resolution denitrification kinetics in pasture soils link N_2O emissions to pH, and denitrification to C mineralization. *PLoS ONE* **2016**, *11*, e0151713. [CrossRef] [PubMed]

24. Senbayram, M.; Chen, R.; Mühling, K.H.; Dittert, K. Contribution of nitrification and denitrification to nitrous oxide emissions from soils after application of biogas waste and other fertilizers. *Rapid Commun. Mass Spectrom.* **2009**, *23*, 2489–2498. [CrossRef] [PubMed]

25. Stevens, R.; Laughlin, R. Measurement of nitrous oxide and di-nitrogen emissions from agricultural soils. *Nutr. Cycl. Agroecosyst.* **1998**, *52*, 131–139. [CrossRef]

26. Qu, Z.; Wang, J.; Almøy, T.; Bakken, L.R. Excessive use of nitrogen in Chinese agriculture results in high $N_2O/(N_2O+N_2)$ product ratio of denitrification, primarily due to acidification of the soils. *Glob. Chang. Biol.* **2014**, *20*, 1685–1698. [CrossRef] [PubMed]

27. Khan, S.; Clough, T.; Goh, K.; Sherlock, R. Influence of soil pH on NO_x and N_2O emissions from bovine urine applied to soil columns. *N. Z. J. Agric. Res.* **2011**, *54*, 285–301. [CrossRef]

28. Clough, T.J.; Kelliher, F.M.; Sherlock, R.R.; Ford, C.D. Lime and soil moisture effects on nitrous oxide emissions from a urine patch. *Soil Sci. Soc. Am. J.* **2004**, *68*, 1600–1609. [CrossRef]

29. Mkhabela, M.; Gordon, R.; Burton, D.; Madani, A.; Hart, W. Effect of lime, dicyandiamide and soil water content on ammonia and nitrous oxide emissions following application of liquid hog manure to a marshland soil. *Plant. Soil* **2006**, *284*, 351–361. [CrossRef]

30. Galbally, I.E.; Meyer, C.P.; Wang, Y.-P.; Smith, C.J.; Weeks, I.A. Nitrous oxide emissions from a legume-pasture and the influences of liming and urine addition. *Agric. Ecosyst. Environ.* **2016**, *230*, 353. [CrossRef]

31. Page, K.; Allen, D.; Dalal, R.; Slattery, W. Processes and magnitude of CO_2, CH_4, and N_2O fluxes from liming of Australian acidic soils: A review. *Soil Res.* **2010**, *47*, 747–762. [CrossRef]

32. Di, H.; Cameron, K. How does the application of different nitrification inhibitors affect nitrous oxide emissions and nitrate leaching from cow urine in grazed pastures? *Soil Use Manag.* **2012**, *28*, 54–61. [CrossRef]