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Contribution of nitrogenous sources to carbon mineralization and soil health

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Abstract

The non-judicious use of nitrogen (N) fertilizers in agricultural soils increases environmental pollution, greenhouse gases (GHGs) emission, and ammonia (NH_3) volatilization. Nitrogen sources undergo numerous biological and chemical transformations that may positively or negatively impact soil organic carbon (SOC) dynamics and soil health. To determine the impact of various N sources on carbon mineralization, soil health, and NH_3 volatilization in alkaline calcareous soil, an incubation experiment was executed using three N sources i) urea as NH_4-N, ii) calcium nitrate as NO_3-N, and iii) ammonium nitrate as both NH_4- and NO_3-N, and a control without N addition. The results showed that urea addition had the highest C-CO_2 emission and N-NH_3 volatilization compared with other treatments. The microbial biomass carbon (MBC) and metabolic quotient (qCO_2) were increased by all N sources, with significantly higher values (327.76 mg C kg^{-1} soil and 0.32 qCO_2, respectively) observed with urea. The SOC decreased, and β-glucosidase enzyme activity increased with NH_4-N sources. At the same time, the activities of acid phosphatase and leucine aminopeptidase enzymes significantly enhanced with NH_4NO_3, except for chitinase. It was concluded that adding inorganic NH_4-N in alkaline calcareous soils under controlled conditions resulted in higher emissions of CO_2, and NH_3 volatilization due to higher microbial activities. Therefore, the selection of N sources should be based on their potential contribution to GHGs emission, C mineralization, and soil health, which are crucial for sustainable crop production, particularly in alkaline calcareous soils with low SOC.

Keywords: Nitrogen sources; CO_2 emissions; NH_3 volatilization; microbial activities; soil organic carbon; alkaline calcareous soils

Introduction

Efficient N fertilizer management is a central challenge in sustainable agriculture, as various N forms like ammonium (NH_4^+) and nitrate (NO_3^-) have divergent effects on soil processes and crop productivity (Tufail *et al.,* 2024). Because the excessive N fertilizers applied to field crops with varying sources may be lost as volatilization, leaching, nitrification, and denitrification processes in the soil system (Arora and Srivastava, 2013). For sustainable crop production, various organic and mineral nitrogenous fertilizers such as animal manure, urea, diammonium phosphate, calcium ammonium nitrate, and nitrophos are widely used (Hasler *et al.,* 2015). These fertilizers are used to manage agroecosystems for improving soil quality and fertility (Shang *et al.*, 2014). But it may accelerate SOC mineralization, leading to increased CO_2 emissions and potential depletion of long-term soil C storage and soil health (Ashraf et al., 2023). The variety of biological and chemical processes in soil results in a loss of up to 70% of the applied N, primarily through ammonia (NH_3) volatilization, nitrous oxide (N_2O) emissions, and NO_3^- leaching from the soil (Erisman *et al.,* 2008).

Ammonia loss results from the high pH-induced conversion of NH_4^+ to NH_3. The change in soil pH following N fertilizer application may significantly impact the NH_3 volatilization. In alkaline soils, factors such as high temperature, low cation exchange capacity, and light mist may accelerate NH_3 losses (Mikkelsen, 2009). About 56% of global inorganic N fertilizers are produced in the form of urea (Sigurdarson *et al.,* 2018), which is a significant contributor to CO_2, methane and N_2O emissions (Zhang *et al.,* 2013), as well as NH_3 volatilization (Mariano *et al.,* 2019).

Various forms of N fertilizers may influence and accelerate the decomposition of native soil organic matter (SOM) already present in soil by directly affecting soil characteristics, enzyme activities, and microbial biomass

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(Jia *et al.*, 2020). The extracellular enzymes break down SOM into simple organic molecules in response to N addition, which are valuable indicators of decomposition (Carreiro *et al.*, 2000) and are involved in C and N cycling (de Andrade Barbosa *et al.*, 2019). The NH_4-N and NO_3-N sources may have contrasting impacts on microbial activities due to their different ionic charges and sources of origin (Cao *et al.*, 2021). The N ions present in abundance control the SOM decomposition process, and NH_4^+ ions are preferred by microbes relative to NO_3^- due to their lower energy cost (Min *et al.*, 2011). Although many studies have been reported in various ecosystems and soil types, illustrating the rate, forms, and duration of N addition. However, no study has reported the direct impacts of N sources on C mineralization in alkaline calcareous soil conditions. So, it is very important to elucidate how the application of different N sources, i.e., NH_4-N, NO_3-N, and combined NH_4- and NO_3-N, can impact microbial activities and C mineralization as well as NH_3 volatilization in alkaline calcareous soils having low SOM. Based on the facts mentioned above, two hypotheses were generated: 1) NH_4- and NO_3-N sources could have a contrasting impact on SOM mineralization, and 2) NH_4-N and NO_3-N induced pH changes may accelerate microbial activities in low SOC alkaline calcareous soil.

Materials and Methods

Soil sampling and Characterization

For the incubation study, topsoil (0-15 cm) was collected from the research field of the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan (31.4391° N, 73.0700° E). The research field has been under a wheat-maize cropping system for the last 4 years, with the recommended NPK application, and was irrigated with both canal and groundwater. The climate of the site is semi-arid, characterized by a mean annual temperature of approximately 31.8°C, a mean annual rainfall of 101 mm, and a relative humidity ranging from 19% to 48%. After removing visible plant roots and debris, the soil was dried, ground, and finally sieved (2 mm) for basic physical and chemical characterization. The textural class of soil was loam containing sand (42%), silt (38%), and clay (20%). The pH of the saturated soil paste and the electrical conductivity of the extract of soil were 7.9 and 1.41 dS m^{-1}, respectively. The water-holding capacity of the soil (WHC) was 19.50%. The SOC, total soil N content and C:N ratio were 0.57%, 540 mg kg^{-1} and 10.56, respectively. The soil extractable P was 12.91 mg kg^{-1}, and the soil exchangeable K was 104.38 mg kg^{-1}.

Incubation study

For incubation, 100 g of soil was placed in 500 mL incubation jars. After pre-incubation for 1 month, treatments such as N sources: urea as NH_4-N, calcium nitrate as NO_3-N, and ammonium nitrate as both NH_4- and NO_3-N were added at the rate of 5% of total soil N, and moisture was adjusted to 80% WHC by adding distilled water. The control without N addition was also maintained. Four replications of each treatment were maintained in a completely randomized design (CRD).

Gaseous emissions

The gaseous emissions (CO_2 and NH_3) were determined from each incubation jar by using a portable multi-gas analyzer (GT-1000-JM4) after 1, 2, 3, 4, 5, 7, 10, 15, 20, 30, 45 and 60 days of incubation. The incubation jars were flushed with fresh air after every sampling event. The gaseous emissions were calculated according to the formula used by Shaaban *et al.* (2015):

$$F = \frac{(\rho \times V \times IR \times 273 \times AM)}{(Wt. \times Temp. \times MM)} \times \Delta c/\Delta t \times 10$$

Where, F is the emission rate of the respective gas (mg Kg^{-1} soil hr^{-1}); IR is the instrument reading (ppm); ρ is density of a specific gas at standard conditions (g L^{-1}), V is volume of incubation jar (L); 273 is temperature factor for (Kelvin Scale); AM is atomic mass of respective gas atom (g); Wt. is soil weight (g); Temp. is incubation temperature in Kelvin Scale (T^oC + 273); MM is the molecular mass of the respective gas (g); Δc is the gas production during the sealed time of the jar, Δt is the sealed time (hr), and 10 is the conversion factor for kg weight basis of soil.

Microbial biomass carbon

The MBC content in the soil was determined by the chloroform fumigation extraction method (Vance *et al.*, 1987). For this purpose, 10 g of fresh soil was divided into two fractions (5g each for fumigation and non-fumigation treatment) and was extracted with 20 mL of 0.05 M K_2SO_4 solution. The fumigation was performed with ethanol-free $CHCl_3$ for 24 hours. The 4 mL of fumigated and non-fumigated soil extracts, with 5 mL of concentrated H_2SO_4 and 1 mL of $K_2Cr_2O_7$, were titrated after cooling against acidified ferrous ammonium sulfate in the presence of phenanthroline indicator until the appearance of a brick red endpoint. The formula EC/K_{EC} was used to compute the MBC values, where EC is the difference in organic C between fumigated and non-fumigated soil, and K_{EC} is a factor (0.45) for converting microbial flush dissolved into microbial C (Wu *et al.*, 1990). The non-fumigated soil extracted C was regarded as the

dissolved organic carbon (DOC) content. The metabolic quotient ($_qCO_2$) was calculated as the ratio of C-CO_2 emissions to the soil MBC content for each treatment of the experiment (Anderson and Domsch, 1993).

Extracellular enzymes activities

Extracellular enzyme activities from fresh soil samples were determined by using fluorogenically labelled substrates technique (Pritsch *et al.*, 2004; Sanaullah *et al.*, 2011). The three methylumbelliferone (MUF)-based fluorogenic enzyme substrates used were MUF-β-D-glucopyranoside for β-glucosidase enzyme, MUF-N-acetyl-β-D-glucosaminide dehydrate for chitinase enzyme, and MUF-phosphate monoester for acid phosphomonoesterase. The L-Leucine-7-amino-4-methylcoumarin (AMC) substrate was used to determine the L-Leucine aminopeptidase activity.

Briefly, 0.5 g of fresh soil was dispersed in 50 mL of sterile distilled water on a reciprocating shaker at 320 rpm for 30 minutes. Then 50 µL of soil suspension was pipetted into a 96-well microplate containing 50 µL of buffer solutions, such as 2-(N-morpholino) ethanesulfonic acid (MES) salt for MUF enzymes (β-Glucosidase, Chitinase and Acid Phosphatase) and *Tris(hydroxymethyl)aminomethane* (TRIZMA)-Base and TRIS-HCl salts for AMC substrates enzyme (Leucine- Aminopeptidase). After that, 100 µL of respective substrates of each enzyme were added, making the final concentration of 200 µmol g^{-1} soil in each well. After pipetting all these, the soil suspensions were incubated with fluorogenic substrates for 2 hours at room temperature. The fluorescence at an excitation/emission wavelength of 360/460 nm was measured using a multilabel microplate reader (Synergy, Biotek, USA). The enzyme activities were expressed as MUF or AMC released in nMol g^{-1} h^{-1}.

Soil chemical properties

The pH of the soil samples was determined using a 1:1 soil-to-distilled water ratio suspension (Thomas, 1996) with a pH meter (OHAUS ST230 pH).

For total soil N, soil samples were digested in a concentrated sulfuric acid solution. Specifically, 0.5 g of soil was mixed with 10 mL of H_2SO_4 and three drops of H_2O_2 in a microwave block digester (MDS-6G, SINEO Microwave Chemistry Technology, Shanghai, China) at 200°C for 2 hours. The total soil N from digested soil samples was determined using an automatic Kjeldahl distillation unit (K9840, Hannon Instruments, Shanghai, China), following the method of Jackson (1964). The distillate collected in a 50-mL digestion flask was titrated against 0.01N H_2SO_4 till the appearance of pink colour in the presence of mixed indicators (bromocresol green and methyl-red). The titration volume was recorded, and the total N in soil was calculated.

The SOC contents were determined by the modified wet oxidation Walkley-Black method (Nelson and Sommers, 1983). Briefly, the 0.5 g air-dried soil samples were treated with 5 mL $K_2Cr_2O_7$ and 10 mL concentrated H_2SO_4 and diluted with distilled water to 100 mL volume. The 5 mL of concentrated H_3PO_4 was added, and after cooling for 30 minutes it was titrated against 0.5 M ferrous ammonium sulfate solution until green endpoint in the presence of diphenylamine indicator. The batch of blank samples, without soil and containing all the reagents, was also treated the same as the sample, and SOC was calculated.

Statistical analysis

The experimental design was a simple CRD for studying individual impacts of different N sources on the studied parameters. One-way analysis of variance followed by the Least Significant Difference test was used to assess significant differences ($p \leq 0.05$) between treatment means using the analytical software Statistix (*v* 8.1). Pearson's correlation analysis was performed to find the possible relationships among the parameters studied using the packages "corrplot" and "agricolae" in R software (*v* 4.2.0). The violin graphs of data were made by using the GraphPad Prism software (*v* 10.0.2.)

Results

Gaseous emissions

The cumulative C-CO_2 emissions significantly increased by the addition of all N sources and were highest in NH_4-forms of N addition i.e. urea addition has the highest C-CO_2 emissions (106.28 mg C-CO_2 kg^{-1} soil) followed by combined NH_4NO_3 (93.79 mg C-CO_2 kg^{-1} soil), calcium nitrate (38.24 mg C-CO_2 kg^{-1} soil) and no N (20.76 mg C-CO_2 kg^{-1} soil) (Figure 1a and 1b). The soil C-CO_2 efflux was highest during the first three days for all the treatments. The C-CO_2 efflux was highest by NH_4-N sources, the maximum (0.449 mg kg^{-1} soil h^{-1}) was observed in combined NH_4NO_3 (Figure 1c).

Similar to C-CO_2 emissions, the cumulative N-NH_3 emissions significantly increased by the addition of all N sources as compared to the control. The urea addition had the highest N-NH_3 emissions (598 µg N-NH_3 kg^{-1} soil) compared with ammonium nitrate (394 µg N-NH_3 kg^{-1} soil) and calcium nitrate (73 µg N-NH_3 kg^{-1} soil) (Figure 2a and 2b). The NH_4-N sources had the highest efflux rate as (5.5 µg kg^{-1} soil h^{-1}) observed in ammonium nitrate and (3.34 µg kg^{-1} soil h^{-1}) in urea till day 10 (Figure 2c).

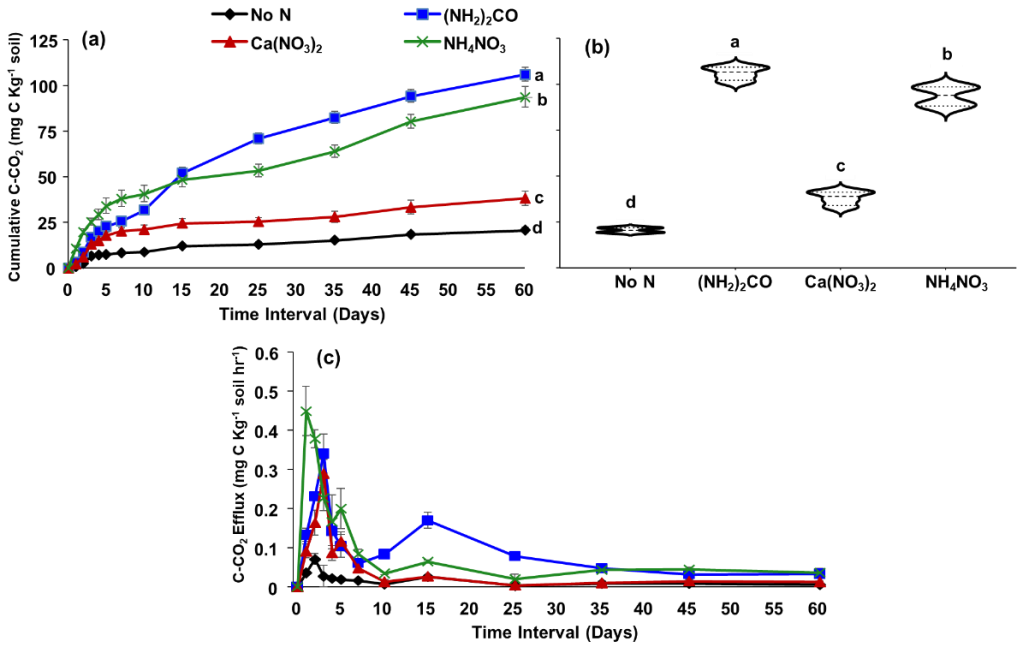


Figure 1: Influence of different N sources (no N, urea, calcium nitrate and ammonium nitrate) addition on a) & b) Cumulative C-CO_2 emissions (mg kg^{-1} soil) and c) C-CO_2 efflux (mg kg^{-1} soil h^{-1}). Data Represented as mean ± SD (n=4). The violin graphs indicate mean and quartile values of the data

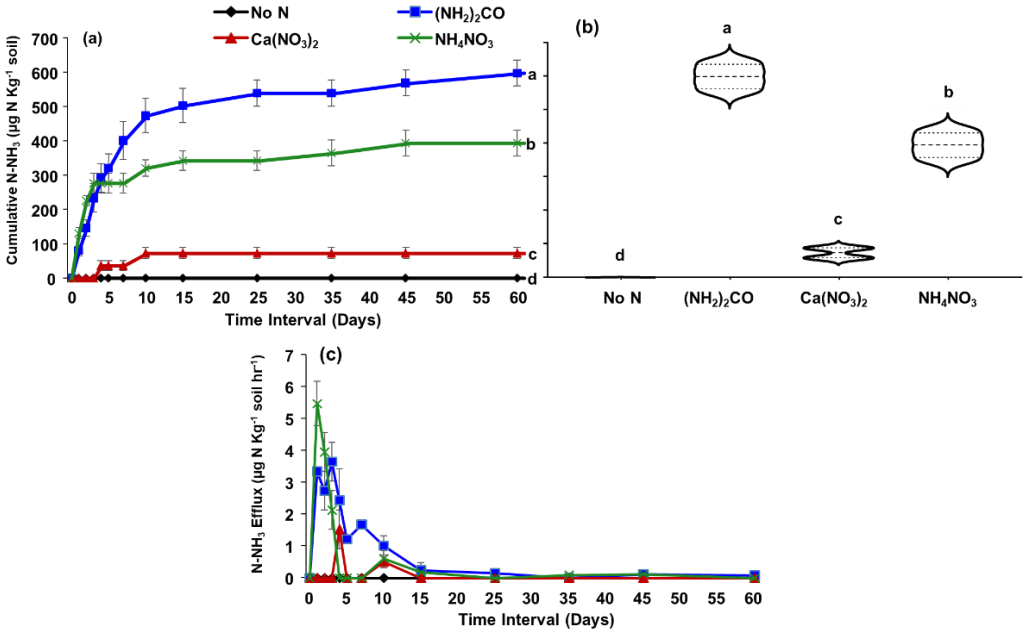


Figure 2: Influence of different N sources (no N, urea, calcium nitrate and ammonium nitrate) application on a) & b) Cumulative N-NH_3 volatilization (µg N kg^{-1} soil) and c) N-NH_3 efflux (µg N kg^{-1} soil h^{-1}). Data Represented as mean ± SD (n=4). The violin graphs indicate mean and quartile values of the data.

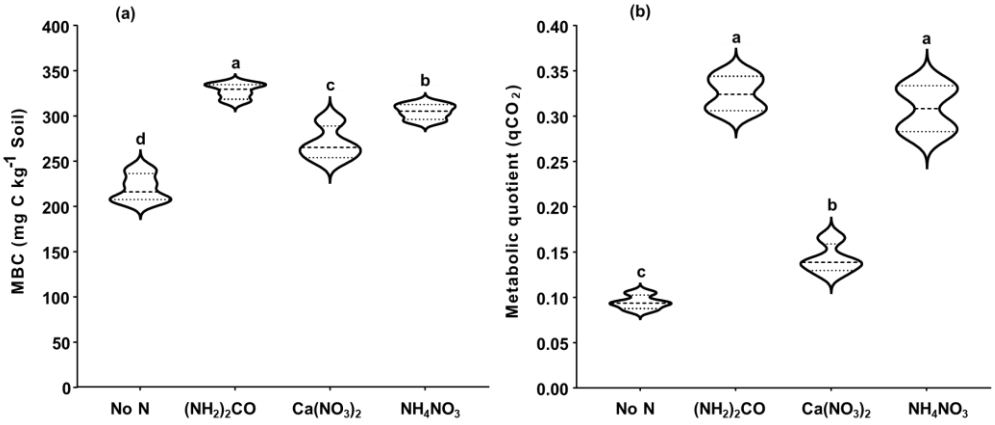


Figure 3: Impact of different N sources (no N, urea, calcium nitrate and ammonium nitrate) on a) Microbial biomass carbon (mg C kg^{-1} soil) and b) Metabolic quotient (qCO_2). The violin graphs indicate mean and quartile values of the data

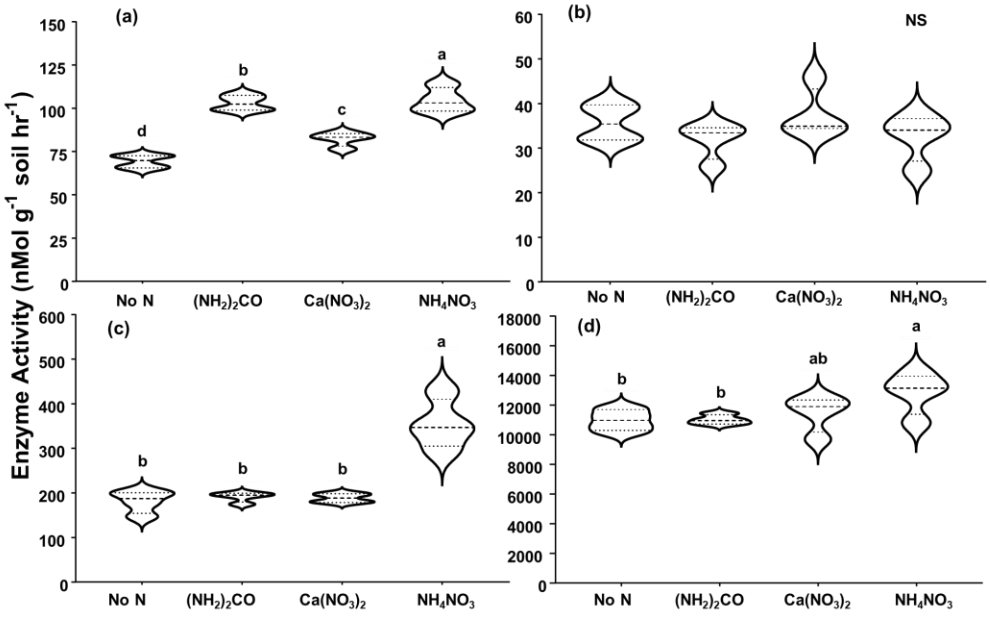


Figure 4: Extracellular enzymes activities (nMol g^{-1} soil h^{-1}) a) β-glucosidase, b) Chitinase, c) Acid phosphatase, and d) Leucine aminopeptidase as affected by different N sources (no N, urea, calcium nitrate and ammonium nitrate). The violin graphs indicate mean and quartile values of the data

Microbial biomass carbon and Metabolic quotient

The addition of all N sources significantly ($p \leq 0.05$) increased the soil MBC, but it was significantly higher in NH_4-N addition, and the order was as urea > NH_4NO_3 > $Ca(NO_3)_2$. The maximum MBC was in urea (327.76 mg C kg^{-1} soil), and the minimum was in no N (220.08 mg C kg^{-1} soil) (Figure 3a).

Similarly, the metabolic quotient was higher in NH_4-N forms of urea and ammonium nitrate compared to the $Ca(NO_3)_2$. The metabolic quotient was increased (77.87, 69.32, and 33.56%) by urea, ammonium nitrate, and calcium nitrate, respectively (Figure 3b).

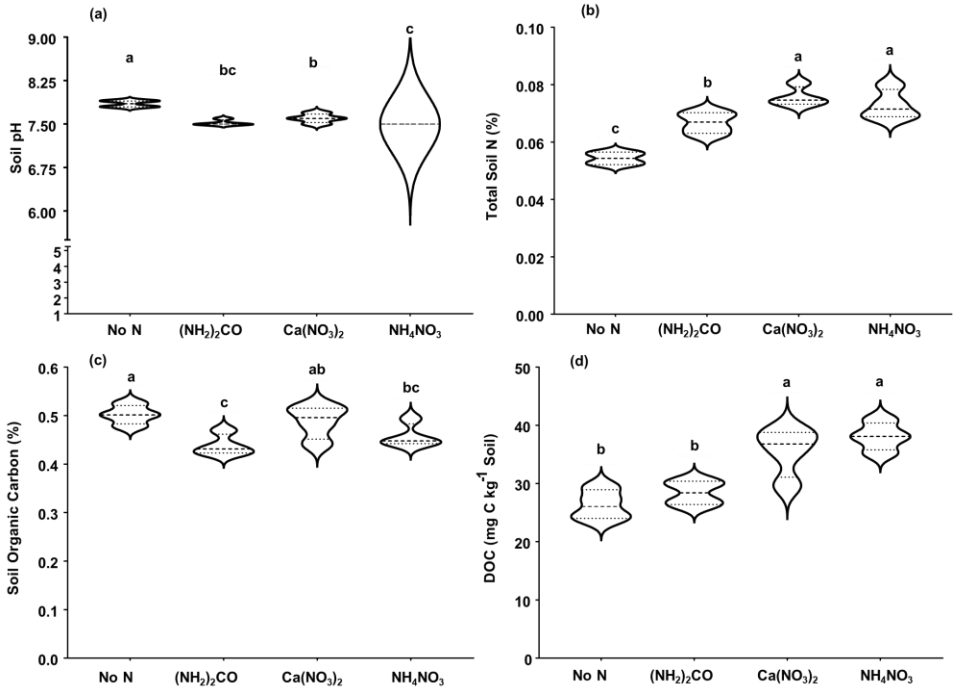
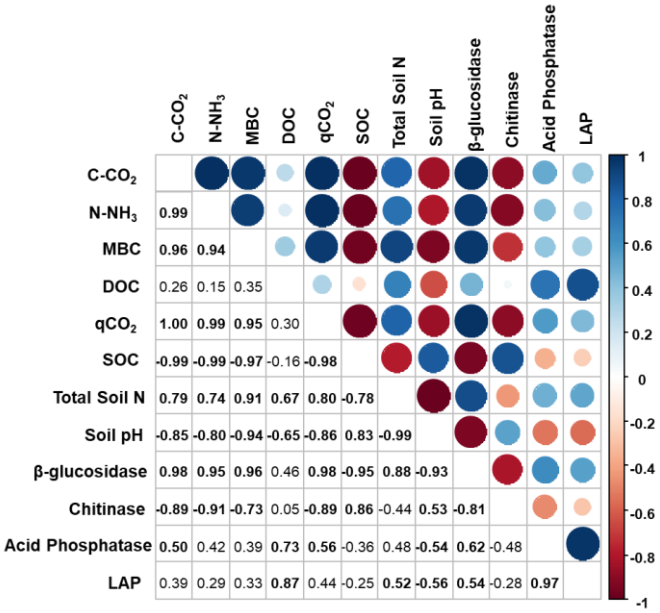


Figure 5: Impact of different N sources (no N, urea, calcium nitrate, and ammonium nitrate) on a) Soil pH, b) Total soil nitrogen (%), c) Soil organic carbon (%), and d) Dissolved organic carbon (mg C kg^{-1} soil). The violin graphs indicate the mean and quartile values of the data.



	C-CO_2	N-NH_3	MBC	DOC	qCO_2	SOC	Total Soil N	Soil pH	β-glucosidase	Chitinase	Acid Phosphatase	LAP
C-CO_2												
N-NH_3	0.99											
MBC	0.96	0.94										
DOC	0.26	0.15	0.35									
qCO_2	1.00	0.99	0.95	0.30								
SOC	-0.99	-0.99	-0.97	-0.16	-0.98							
Total Soil N	0.79	0.74	0.91	0.67	0.80	-0.78						
Soil pH	-0.85	-0.80	-0.94	-0.65	-0.86	0.83	-0.99					
β-glucosidase	0.98	0.95	0.96	0.46	0.98	-0.95	0.88	-0.93				
Chitinase	-0.89	-0.91	-0.73	0.05	-0.89	0.86	-0.44	0.53	-0.81			
Acid Phosphatase	0.50	0.42	0.39	0.73	0.56	-0.36	0.48	-0.54	0.62	-0.48		
LAP	0.39	0.29	0.33	0.87	0.44	-0.25	0.52	-0.56	0.54	-0.28	0.97	

*LAP= leucine aminopeptidase

Figure 6: Pearson correlation coefficients among measured variables with the addition of different N sources (no N, urea, calcium nitrate, and ammonium nitrate)

Soil extracellular enzymes activities

Among extracellular enzymes, β-glucosidase activity was significantly ($p \leq 0.05$) increased by the addition of N sources and was maximum with the addition of combined sources, i.e., NH_4NO_3 (104.44 nMol g^{-1} h^{-1}), followed by single NH_4-N and NO_3-N sources (Figure 4a). There was no significant impact of N sources addition on all other three determined enzymes (chitinase, acid phosphatase, and LAP), except increased activity of acid phosphatase enzyme (354.05 nMol g^{-1} h^{-1}) and LAP (12825 nMol g^{-1} h^{-1}) with the addition of NH_4NO_3 (Figure 4b, c and d).

Soil chemical properties

The addition of different N sources slightly decreased soil pH, and this reduction was higher with the addition of NH_4-forms of N (Figure 5a). The total soil N was substantially higher where NO_3-N was added, either individually at 0.076% and combined NH_4NO_3 at 0.073% (Figure 5b).

The SOC contents were significantly ($p \leq 0.05$) decreased when N was applied as NH_4-N either individually (urea) or in combined form (ammonium nitrate). The change in SOC contents with the application of NO_3-N source was non-significant compared with the control having no N (Figure 5c). The DOC contents followed a similar trend to that of total soil N, with DOC contents being significantly higher where NO_3-N was added (Figure 5d).

Correlations

The Pearson correlation analysis (Figure 6) showed that C-CO_2 emissions were positively correlated with qCO_2 (1.00), β-glucosidase (r = 0.98), MBC (r = 0.96), total soil N (r = 0.79), and acid phosphatase (r = 0.50). But CO_2 emissions were negatively influenced by SOC (r = -0.99), chitinase (r = -0.89) and soil pH (r = -0.85). The NH_3 emissions illustrated a positive relationship with qCO_2 (r = 0.99), MBC (r = 0.94), β-glucosidase (r = 0.89), and total soil N (r = 0.74) while a negative relationship with SOC (r = -0.99), chitinase (r = -0.91) and soil pH (r = -0.80).

Discussion

The application of various N sources enhanced microbial activities, which in turn increased SOC mineralization and higher CO_2 emissions compared to the no-N treatment (Russell *et al.*, 2009; Anjum and Khan, 2021). The applied N may alleviate N limitation to soil microbes, allowing them to decompose organic matter more efficiently due to the positive priming effect (Wild *et al.*, 2014; Averill and Waring, 2018). The negative relationship between C-CO_2 emissions and SOC (r = -0.99) indicated that with SOM decomposition, SOC decreased and CO_2 increased. The soil N application can induce changes in the composition, diversity, abundance, and functional potential of soil microbial communities to higher carbon-demanding microbial groups that contribute more to MBC (Wu *et al.*, 2021; Bei *et al.*, 2022). Furthermore, the N addition promotes the growth, reproduction, and metabolic activity of microbes and alters N cycling processes, which enhance SOM decomposition and, in return, increase soil MBC (Guo *et al.*, 2022; Elrys *et al.*, 2023) as correlated with total soil N and MBC (r = 0.91). The increase in soil total N content may be due to N buildup from applied sources or the release of more N to the soil by higher SOM decomposition (Pahalvi *et al.*, 2021).

The higher C-CO_2 emissions with the application of NH_4-N (especially urea) might be because of microbial preference for NH_4-N being a low-energy cost source for their activity (Puri and Ashman, 1999; Min *et al.*, 2011) or by decreased soil pH during the conversion of ammonium to nitrate during microbial oxidation, and is correlated with the changes in soil pH and C-CO_2 (r = -0.85). This finding is consistent with the second hypothesis that NH_4-N and NO_3-N induced pH changes may accelerate microbial activity in low SOC alkaline calcareous soils. Such a pH change stimulates the biomass and activity of microbial communities, involving processes that affect C cycling and CO_2 emissions (Kunhikrishnan *et al.*, 2016). This is also illustrated by the positive relationship between C-CO_2 and MBC (r = 0.96), indicating microbial biomass production. Additionally, there is a positive relationship between C-CO_2 and qCO_2 (r = 1.00) and between C-CO_2 and β-glucosidase (r = 0.98), indicating that microbial activity is present. Another reason is that applied NH_4-N may potentially enhance the priming effect of soil labile C to stimulate the mineralization of existing SOM (Mason-Jones *et al.*, 2018) and lead to enhanced microbial mining of N from stable organic matter decomposition (Murphy *et al.*, 2015; Hicks *et al.*, 2021). Similarly, the enhanced cumulative N-NH_3 volatilization with the application of ammoniacal N sources (urea & ammonium nitrate) might be due to the hydrolysis reaction of urease enzyme already present in soil, converting urea into ammonium carbonate that is unstable and rapidly breaks down into NH_3 and CO_2 (Erisman *et al.*, 2008; Fenn and Hossner, 2012). This is illustrated by the positive relationship between N-NH_3 emissions and soil total N (r = 0.74). The ammoniacal sources in high-pH alkaline soils favor the conversion of NH_4^+ to NH_3, which has high volatility (Sigunga *et al.*, 2002; Rochette *et al.*, 2013). The lowest emission of NH_3 with calcium nitrate might be attributed to its highly oxidized and stable form, as it does not undergo hydrolysis (Cameron *et al.*, 2013). The higher MBC contents with the application of NH_4-N sources might be due to the readily available N, which

serves as a nutrient source for microbial activity (Yue *et al.*, 2016). The reduced SOC and higher CO_2 emissions by NH_4^+ sources compared with NO_3^- showed that nitrate could have an inhibitory impact on SOC decomposition (Su et al., 2024). This is consistent with the first hypothesis that NH_4 and NO_3-N sources could have contrasting impacts on SOM mineralization and CO_2 emissions. In the limited C supply, as observed in this study, the N supply indirectly facilitates MBC by providing microbes with C substrates through the decomposition of SOM (Raza *et al.*, 2023), which is highly correlated with β-glucosidase and MBC (r = 0.96). The enhanced DOC by calcium nitrate and ammonium nitrate, which were insignificant with urea, were inconsistent with the findings of Liu and Greaver (2010). Moreover, Yue *et al.* (2016) found a 11.67% increase in DOC by ammonium nitrate and 29.77% by nitrate sources, and insignificant with ammonium N, as correlated by total soil N and DOC (r = 0.67).

The increased activity of β-glucosidase enzyme by N sources might be due to the decreased C:N ratio of soil, which accelerated microbes to produce more C-cycle enzymes relative to N-cycle enzymes, as β-glucosidase is involved in the C cycling (Chen *et al.*, 2014; de Almeida *et al.*, 2015). The application of NH_4-N form may enhance the microbial decomposition of SOM to release more β-glucosidase in the soil (Dong *et al.*, 2020; Jia *et al.*, 2020), as illustrated by the negative correlation of β-glucosidase with SOC (r = -0.95). The increased activity of acid phosphatase under NH_4-N form application may be due to the reduced pH of the soil, which enhances microbial activity for the breakdown of organic matter and the release of organically bound P in the soil (Olander and Vitousek, 2000; Marklein and Houlton, 2012).

The slight decrease in soil pH resulting from different N sources, especially NH_4-N forms, may be attributed to the generation of H+ ions during microbial oxidation of NH_4^+ to NO_3^- or to a decrease in the saturation of exchangeable base cations (Ca^{2+} and Mg^{2+}) in the soil (Fageria *et al.*, 2010). The higher soil total N content from calcium nitrate and ammonium nitrate sources may be attributed to reduced volatilization losses (Vangeli *et al.*, 2022) and the absence of leaching losses in the soil incubation study.

Conclusion

The NH_4-N addition, either alone or in combination (urea and ammonium nitrate), enhanced microbial activities, which resulted in higher cumulative C-CO_2 and N-NH_3 emissions compared with NO_3-N sources. In addition, urea as N source caused a decrease in SOC contents of alkaline calcareous soil. The right form of N should be considered in conjunction with the rate, time, and place of N application for sustainable crop production. This study did not account for the effects of vegetation, plant N uptake, root exudates, and aboveground biomass inputs, which play important roles in shaping overall soil health and could therefore influence the results. Hence, prospective studies should include the following: i) field experiments carried out in different cropping systems; ii) N forms influence on bulk and rhizospheric soil organic matter variations; and iii) the co-effect of crop litter and N input on SOM transformations and soil health.

Authors' contribution statement

Ahmad Mujtaba: Experimentation, Writing- Original draft; Abdul Wakeel: Data curation, Conceptualization, Revision; Muhammad Sanaullah: Visualization, Investigation, Supervision, Data Validation and Writing- Reviewing and Editing.

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References

Anderson, T.H. and K.H. Domsch. 1993. The metabolic quotient for CO_2 (qCO_2) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soils. *Soil Biology and Biochemistry* 25: 393-395.

Anjum and A. Khan. 2021. Decomposition of soil organic matter is modulated by soil amendments. *Carbon Management* 12: 37-50.

Arora, K. and A. Srivastava. 2013. Nitrogen losses due to nitrification: Plant based remedial prospects. *International Journal of Bioassays* 2: 984-991.

Ashraf, M.N., C. Hu, X. Xu, T. Aziz, L. Wu, M.A. Waqas, M. Farooq, X. Hu, W. Zhang and M. Xu. 2023. Long-term manure application increased soil organic carbon and nitrogen mineralization through accumulation of unprotected and physically protected carbon fractions. *Pedosphere 33*: 343-354.

Averill, C. and B. Waring. 2018. Nitrogen limitation of decomposition and decay: How can it occur? *Global Change Biology* 24: 1417-1427.

Bei, S., X. Li, T.W. Kuyper, D.R. Chadwick and J. Zhang. 2022. Nitrogen availability mediates the priming effect of soil organic matter by preferentially altering the straw carbon-

assimilating microbial community. *Science of the Total Environment* 815: e152882.

Cameron, K.C., H.J. Di and J.L. Moir. 2013. Nitrogen losses from the soil/plant system: A review. *Annuals of Applied Biology* 162: 145-173.

Cao, Y., Z. He, T. Zhu and F. Zhao. 2021. Organic-C quality as a key driver of microbial nitrogen immobilization in soil: A meta-analysis. *Geoderma* 383: e114784.

Carreiro, M., R. Sinsabaugh, D. Repert and D. Parkhurst. 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. *Ecology* 81: 2359-2365.

Chen, R., M. Senbayram, S. Blagodatsky, O. Myachina, K. Dittert, X. Lin, E. Blagodatskaya and Y. Kuzyakov. 2014. Soil C and N availability determine the priming effect: microbial N mining and stoichiometric decomposition theories. *Global Change Biology* 20: 2356-2367.

de Almeida, R.F., E.R. Naves and R.P. da Mota. 2015. Soil quality: Enzymatic activity of soil β-glucosidase. *Global Journal of Agricultural Research and Reviews* 3: 146-450.

de Andrade Barbosa, M., R.L. de Sousa Ferraz, E.L.M. Coutinho, A.M.C. Neto, M.S. da Silva,C. Fernandes and E.C. Rigobelo. 2019. Multivariate analysis and modeling of soil quality indicators in long-term management systems. *Science of the Total Environment* 65: 7457-465.

Dong, L., B. Berg, T. Sun, Z. Wang and X. Han. 2020. Response of fine root decomposition to different forms of N deposition in a temperate grassland. *Soil Biology and Biochemistry* 147: e107845.

Elrys, A.S., Q. Zhu, C. Jiang, J. Liu, H.H. Sobhy, Q. Shen, Y. Uwiragiye, Y. Wu, K.A. El-Tarabily and L. Meng. 2023. Global soil nitrogen cycle pattern and nitrogen enrichment effects: Tropical versus subtropical forests. *Global Change Biology* 29: 1905-1921.

Erisman, J.W., M.A. Sutton, J. Galloway, Z. Klimont and W. Winiwarter. 2008. How a century of ammonia synthesis changed the world. *Nature Geoscience* 1: 636-639.

Fageria, N., A. Dos Santos and M. Moraes. 2010. Influence of urea and ammonium sulfate on soil acidity indices in lowland rice production. *Communications in Soil Science and Plant Analysis* 41: 1565-1575.

Fenn, L. and L. Hossner. Ammonia Volatilization from Ammonium or Ammonium-Forming Nitrogen Fertilizers. 2012. p. 123-169. In: Advances in Soil Science. B.A. (eds.). Springer, New York.

Guo, P., Stewart, L. Yang, D. Kong and H. Zhao. 2022. Differential effects of ammonium and nitrate addition on soil microbial biomass, enzymatic activities, and organic carbon in a temperate forest in North China. *Plant and Soil* 481: 595-606.

Hasler, K., S. Bröring, S. Omta and H.W. Olfs. 2015. Life cycle assessment (LCA) of different fertilizer product types. *European Journal of Agronomy* 69: 41-51.

Hicks, L.C., K. Lajtha and J. Rousk. 2021. Nutrient limitation may induce microbial mining for resources from persistent soil organic matter. *Ecology* 102: e03328.

Jackson, M. 1964. Chemical composition of soils. p. 498. In: Chemistry of the soil. Firman, E.B. (eds.). Reinhold Publications Corporation, New York.

Jia, X., Y. Zhong, J. Liu, G. Zhu, Z. Shangguan and W. Yan. 2020. Effects of nitrogen enrichment on soil microbial characteristics: From biomass to enzyme activities. *Geoderma* 366: e114256.

Kunhikrishnan, A., R. Thangarajan, N. Bolan, Y. Xu, S. Mandal, D. Gleeson, B. Seshadri, M. Zaman, L. Barton and C. Tang. 2016. Functional relationships of soil acidification, liming, and greenhouse gas flux. p. 1-71. In: Advances in Agronomy. Sparks, D.L. (eds.). Academic Press, Burlington.

Liu, L. and T.L. Greaver. 2010. A global perspective on belowground carbon dynamics under nitrogen enrichment. *Ecology Letters* 13: 819-828.

Mariano, E., C.R. de Sant Ana Filho, R. Bortoletto-Santos, J.A. Bendassolli and P.C. Trivelin. 2019. Ammonia losses following surface application of enhanced-efficiency nitrogen fertilizers and urea. *Atmospheric Environment* 203: 242-251

Marklein, A.R. and B.Z. Houlton. 2012. Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. *New Phytologist* 193: 696-704.

Mason-Jones, K., N. Schmücker and Y. Kuzyakov. 2018. Contrasting effects of organic and mineral nitrogen challenge the N-Mining Hypothesis for soil organic matter priming. *Soil Biology and Biochemistry* 124: 38-46.

Mikkelsen, R. 2009. Ammonia emissions from agricultural operations: Fertilizer. *Better Crops* 93: 9-11.

Min, K., H. Kang and D. Lee. 2011. Effects of ammonium and nitrate additions on carbon mineralization in wetland soils. *Soil Biology and Biochemistry* 43: 2461-2469.

Murphy, C.J., E.M. Baggs, N. Morley, D.P. Wall and E. Paterson. 2015. Rhizosphere priming can promote mobilisation of N-rich compounds from soil organic matter. *Soil Biology and Biochemistry* 81: 236-243.

Nelson, D.A. and L.E. Sommers. 1983. Total carbon, organic carbon, and organic matter. p. 539-579. In: Methods of soil analysis: Part 2 Chemical and Microbiological Properties. A.L. Page, (eds.). The American Society of Agronomy, Inc., Soil Science Society of America, Inc., New York.

Olander, L.P. and P.M. Vitousek. 2000. Regulation of soil phosphatase and chitinase activityby N and P availability. *Biogeochemistry* 49: 175-191.

Pahalvi, H.N., L. Rafiya, S. Rashid, B. Nisar and A.N. Kamili. 2021. Chemical fertilizers and their impact on soil health. p. 1-20. In: Microbiota and Biofertilizers, Vol 2: Ecofriendly Tools for Reclamation of Degraded Soil Environs. Gowhar Hamid Dar, R.A.B., Mohammad Aneesul Mehmood, Khalid Rehman Hakeem (eds.). Springer, Cham, London, UK.

Pritsch, K., S. Raidl, E. Marksteiner, H. Blaschke, R. Agerer, M. Schloter and A. Hartmann. 2004. A rapid and highly sensitive method for measuring enzyme activities in single mycorrhizal tips using 4-methylumbelliferone-labelled fluorogenic substrates in a microplate system. *Journal of Microbiological Methods* 58: 233-241.

Puri, G. and M. Ashman. 1999. Microbial immobilization of 15N-labelled ammonium and nitrate in a temperate woodland soil. *Soil Biology and Biochemistry* 31: 929-932.

Raza, T., M.F. Qadir, K.S. Khan, N.S. Eash, M. Yousuf, S. Chatterjee, R. Manzoor, S. ur Rehman and J.N. Oetting. 2023. Unrevealing the potential of microbes in decomposition of organic matter and release of carbon in the ecosystem. *Journal of Environmental Management* 344: e118529.

Rochette, P., D.A. Angers, M.H. Chantigny, M.O. Gasser, J.D. MacDonald, D.E. Pelster and N. Bertrand. 2013. NH_3 volatilization, soil concentration and soil pH following subsurface banding of urea at increasing rates. *Canadian Journal of Soil Science* 93: 261-268.

Russell, A.E., C.A. Cambardella, D.A. Laird, D.B. Jaynes and D.W. Meek. 2009. Nitrogen fertilizer effects on soil carbon balances in Midwestern US agricultural systems. *Ecological Applications*, 19: 1102-1113.

Sanaullah, M., E. Blagodatskaya, A. Chabbi, C. Rumpel and Y. Kuzyakov. 2011. Drought effects on microbial biomass and enzyme activities in the rhizosphere of grasses depend on plant community composition. *Applied Soil Ecology*, 48: 38-44.

Shaaban, M., Q.-a. Peng, R. Hu, Y. Wu, S. Lin and J. Zhao. 2015. Dolomite application to acidic soils: a promising option for mitigating N_2O emissions. *Environmental Science and Pollution Research* 22:19961-19970.

Shang, Q., N. Ling, X. Feng, X. Yang, P. Wu, J. Zou, Q. Shen and S. Guo. 2014. Soil fertility and its significance to crop productivity and sustainability in typical agroecosystem: a summary of long-term fertilizer experiments in China. *Plant and Soil* 381: 13-23.

Sigunga, D.O., B.H. Janssen and O. Oenema. 2002. Ammonia volatilization from Vertisols. *European Journal of Soil Science* 53: 195-202.

Sigurdarson, J.J., S. Svane and H. Karring. 2018. The molecular processes of urea hydrolysis in relation to ammonia emissions from agriculture. *Reviews in Environmental Science and Bio/Technology* 17: 241-258.

Su, S., Z. Zhang, J. Lin, G. Owens and Z. Chen. 2024. How nitrate and ammonium impact soil organic carbon transformation with reference to aggregate size. *Science of The Total Environment 949*: 175213.

Thomas, G. 1996. Soil pH and Soil Acidity. p. 961-1009. In: Methods of Soil Analysis, Part 3-Chemical Methods. D.L. Sparks, A.L. Page, P.A. Helmke, R.H. Loeppert, P.N. Soltanpour, M.A. Tabatabai, C.T. Johnston, M.E. Sumner (eds.). Soil Science Society of America, Madison, WI.

Tufail, M.A., M. Ayyub, L. Tariq, J. Iltaf, A. Asbat, I. Bashir and W. Umar. 2024. Nitrogen fertilizers and the future of sustainable agriculture: a deep dive into production, pollution, and mitigation measures. *Soil Science and Plant Nutrition 70*:457-477.

Vance, E.D., P.C. Brookes and D.S. Jenkinson. 1987. An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry* 19: 703-707.

Vangeli, S., G. Posse, M.E. Beget, E. Otero Estrada, R.A. Valdettaro, P. Oricchio, M. Kandus and C.M. Di Bella. 2022. Effects of fertilizer type on nitrous oxide emission and ammonia volatilization in wheat and maize crops. *Soil Use and Management* 38: 1519-1531.

Wild, B., J. Schnecker, R.J.E. Alves, P. Barsukov, J. Bárta, P. Čapek, N. Gentsch, A. Gittel, G. Guggenberger and N. Lashchinskiy. 2014. Input of easily available organic C and N stimulates microbial decomposition of soil organic matter in arctic permafrost soil. *Soil Biology and Biochemistry* 75: 143-151.

Wu, H., A. Cai, T. Xing, S. Huai, P. Zhu, M. Xu and C. Lu. 2021. Fertilization enhances mineralization of soil carbon and nitrogen pools by regulating the bacterial community and biomass. *Journal of Soils and Sediments* 21: 1633-1643.

Wu, J., R. Joergensen, B. Pommerening, R. Chaussod and P. Brookes. 1990. Measurement of soil microbial biomass C by fumigation-extraction-an automated procedure. *Soil Biology and Biochemistry* 22: 1167-1169.

Yue, K., Y. Peng, C. Peng, W. Yang, X. Peng and F. Wu. 2016. Stimulation of terrestrial ecosystem carbon storage by nitrogen addition: a meta-analysis. *Scientific Reports*, 6: e19895.

Zhang, W.F., Z.X. Dou, P. He, X.T. Ju, D. Powlson, D. Chadwick, D. Norse, Y.L. Lu, Y. Zhang and L. Wu. 2013. New technologies reduce greenhouse gas emissions from nitrogenous fertilizer in China. *Proceedings of the National Academy of Sciences* 110: 8375-8380.