**Effects of Long-term Fertilization History and Current N and S Fertilizer Applications on Nitrous Oxide Production from S-deficient Soils in a Laboratory Incubation**

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Effects of Long-term Fertilization History, and Current N and S Fertilizer Applications on Nitrous Oxide Production from S-deficient Soils in a Laboratory Incubation

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Abstract

The nitrous oxide (N\(_2\)O) production in four soils with unique fertilization management histories - collected from long-term fertility treatments receiving no fertilizer, NPKS, PKS, and NPK in a 5-year cereal-forage rotation - in response to 3 sources of added N (100 kg N ha\(^{-1}\) urea, NH\(_4\)Cl, Ca(NO\(_3\))\(_2\)) with and without co-addition of elemental S (20 kg S ha\(^{-1}\)) plus a 0-N and 0-S control were investigated in a 7-week laboratory incubation in a loam-textured soil at 40% water-filled pore space. In all soils, cumulative N\(_2\)O emissions and apparent, cumulative, net nitrification were significantly higher following addition of urea compared to other N fertilizers with and without co-addition of elemental S. Lower N\(_2\)O emissions were observed in soils without a history of long-term N fertilization following addition of urea compared to soils that had historically received urea. Because the pre-incubation soil total N levels were similar in soils with a history of urea application (NPKS) and without urea (PKS), the results of this investigation suggest that the higher N\(_2\)O production in the NPKS soil may be the result of a priming effect and/or changes in microbial community composition induced by long-term urea applications rather than differences in the long-term soil N balance.
Key words: Hydroxylamine pathway, Long-term fertilization, Nitrification, Nitrifier denitrification, Nitrous oxide, Sulfur oxidation,
Introduction

Increases in crop production in the Canadian Prairie Provinces over the last 30 years have corresponded to increased fertilizer applications. Between 1980 and 2011, wheat and canola yields in the Prairie Provinces increased 43 and 56%, respectively (Graf 2013) and shipments of fertilizer nitrogen (N) from manufacturers to retailers (a proxy for agricultural N applications) doubled from 1 to 2 million metric tonnes over the same period (Dorff and Beaulieu 2014). In recent years, about 50% of total fertilizer N shipments from manufacturers to retailers in the Prairie Provinces has been in the form of urea (Statistics Canada 2016).

Nitrous oxide ($\text{N}_2\text{O}$) is produced as a by-product of urea transformations in soil following its application. Following application, urea undergoes hydrolysis to ammonium which may then be nitrified and $\text{N}_2\text{O}$ is produced during nitrification via the hydroxylamine pathway, nitrifier denitrification and denitrification (Wrage et al. 2001; Braker and Conrad 2011; Stein 2011; Butterbach-Bahl et al. 2013; Jeuffroy et al. 2013; Siciliano 2013). Thus, an increase in urea applications to sustain increasing crop yields have likely increased soil $\text{N}_2\text{O}$ emissions, but changes in management that have coincided with the observed increase in N fertilization (i.e., wide-spread adoption of zero-till) may have mitigating effect. Results from long-term experiments have shown that the contribution of urea transformations to $\text{N}_2\text{O}$ emissions in cultivated soils can be influenced by long-term soil management practices (Skiba and Smith 2000; Drury et al. 2008; Li-mei et al. 2011; LaHue et al. 2015).

In the Parkland region of the Canadian Prairies, sulfur (S) is the most limiting plant nutrient in crops next to N and phosphorus (P) (Malhi et al. 2004). Increased diversity in cropping rotations in the Prairie provinces that include high S-demanding pulse and oilseed crops has apparently
resulted in an increase in S fertilization with total fertilizer S shipments from manufacturers to retailers increasing approximately 5-7% per year between 2012 and 2015 with total shipments reaching 270 000 metric tonnes in 2015 (Statistics Canada 2016).

Elemental S is a concentrated S fertilizer, that is converted to plant-available sulfate (SO$_4^{2-}$) in the soil by S-oxidizing bacteria. There is potential for interaction of N and S cycling in soils through sulfur-driven autotrophic denitrification, the chemolithotrophic process coupling denitrification with the oxidation of reduced inorganic S compounds (Chao 1967; Shao et al. 2010). Denitrification is most often carried out by heterotrophic bacteria, but S-denitrification is carried out autotrophic S-oxidizing bacteria that use N oxides [nitrite (NO$_2^-$), nitrate (NO$_3^-$)] as electron acceptors in oxygen limited conditions. *Thiobacillus denitrificans* is an example of an S-oxidizing autotrophic denitrifier that has been found in soil (Shao et al. 2010). Another possible influence of elemental S on soil N$_2$O emissions is that the oxidation of S decreases soil pH (Modaihsh et al. 1989; Wang et al. 2008) which may increase N$_2$O emissions (Bouwmann 1996).

Although the potential interaction between the N and S transformation processes in the soil may influence N$_2$O emissions, past studies have generally not evaluated the impact of the long-term combined application of N and S fertilization on N$_2$O emissions in S-deficient soils with respect to other associated soil management history (crop rotation and liming). Therefore, a laboratory incubation experiment was designed with the following main objectives: (1) Assess the effects of soil fertilization history on soil N$_2$O production following the application of a variety of fertilizer N sources; and (2) Assess the influence of co-application of elemental S and fertilizer N on soil N$_2$O emissions with respect to soil fertilization history. We expected that N$_2$O emission potential of laboratory incubated soils would be significantly influenced by long-term fertilization history, source of fertilizer N and co-application of elemental S (S$^0$).
MATERIAL AND METHODS

Soils for Incubation

The soils used in this study were collected from the University of Alberta, Breton Classical Plots near Breton, Alberta, Canada (53° 07' N, 114° 28' W). The soils in the Breton Classical Plots are classified as Orthic Gray Luvisol with a loam texture, and an estimated field capacity of 0.25 kg kg⁻¹ or 0.35 m³ m⁻³. The long-term average annual air temperature at the site is 2.1°C, and mean annual precipitation is 547 mm, which mostly occurs between July and August, and the potential evapotranspiration of the site is close to the annual precipitation (Izaurralde et al. 1995). The Breton Classical Plots consist of 8 fertility treatments super-imposed on two rotations: 1) a 2-year wheat (*Triticum aestivum* L.)-fallow (WF) rotation; and 2) a 5-year wheat (*Triticum aestivum* L.)-oats (*Avena sativa*)-barley (*Hordeum vulgare* L.)- alfalfa (*Medicago sativa*)/brome (*Bromus tectorum*) hay (WOBHH) rotation. For this incubation, soils from 4 of the original 8 fertility treatments were sampled from the 5-year rotation and the fertilizer rates for these treatments are given in Table 1.

In 2014, following wheat harvest, soil samples were collected at four random locations in each of the 4 treatment plots using a shovel, from the surface layer of (0-10 cm) limed (east) halves of the following plots of the wheat-oat-barely-hay-hay rotation: (1) Check (2) NPKS (3) NPK (-S) and (4) PKS (-N). Then, after removing the easily detectable crop residues and coarse roots, the samples were air-dried, homogenized, sieved < 2 mm and stored in tin buckets in an insulated storage building until use.

Prior to the incubation experiment, three 90-g sub-samples from each treatment were submitted to the University of Alberta Natural Resources Analytical Laboratory (NRAL) in Edmonton,
Alberta, for analysis of total organic C (TOC), total N (TN), light fraction of C (LFC), light fraction of N (LFN), ammonium-N (NH$_4$-N), nitrate-N (NO$_3$-N), total S (Total S), sulfate-S (SO$_4$$_{2-}$-S) and pH using standard methodology and these properties are summarized in Table 2.

**Experimental Setup**

**Incubation**

The seven-week laboratory incubation experiment (October 8 to November 27, 2015) used a split-plot experimental design with three replicates, which resulted in a complete set of 84 incubation vessels – 1 L mason jars. Main plot treatments were the composite soil samples taken from the Breton Classical Plots treatments: 1) Check (no fertilizer) 2) NPKS 3) NPK 4) PKS.

Subplot treatments (fertilizers) were: (1) nil - no fertilizers; (2) Urea-UR; (3) Ammonium chloride (NH$_4$Cl) - AC; (4) Calcium nitrate (Ca(NO$_3$_)$_2$) - CN; (5) Urea + S$^0$ - UR+S$^0$ (6) Ammonium chloride + S$^0$ - AC+S$^0$; (7) Calcium nitrate + S$^0$ - CN+S$^0$. Rates of N and S were 100 kg N/ha and 20 kg S/ha.

Fertilizer solutions were prepared at concentrations such that 7.5-mL of solution applied to 30-g of soil would achieve N and S (if added) rates of 100 kg N ha$^{-1}$ and 20 kg S ha$^{-1}$ and a water content of 0.25 kg kg$^{-1}$ (40% WFPS).

For all treatments, 30-g of air-dry soil was weighed, packed to a depth of 1 cm at a bulk density of 1 g/cm$^3$ into an ABS cylinder with a sealed bottom and 7.5-mL deionized water for the nil treatment, and 7.5-mL of fertilizer solution for the fertilizer treatments was added using a plastic syringe. The soil sample was then placed inside a 1 L mason jar using forceps. A separate reservoir consisting of 10-mL of tap water was also placed in the jar to help maintain the soil...
moisture content at 0.25 kg kg\(^{-1}\) throughout the incubation period by means of humidity. The moisture content of 0.25 kg kg\(^{-1}\) was chosen because this was the closest representation to the actual field capacity of the soil and this corresponded to 40% water-filled pores space (WFPS).

Following the incubation experiment, the soil in each jar was dried at 60\(^{\circ}\)C for three days and sent to the lab for NO\(_3\)-N, NH\(_4\)-N, and SO\(_4\)^{2-}\)-S analysis according to the methods mentioned previously.

**Gas Sampling**

Gas (CO\(_2\) and N\(_2\)O) concentrations in the jar head space were measured within the first 24 hours following initiation of the experiment, twice per week for the next 3 weeks, and once per week in the remaining four weeks of the experiment with an Innova 1312 photacoustic gas analyzer (Innova Air Tech Instruments, Ballerup, Denmark). The jars remained sealed between sampling times, but following gas sampling, the lids were removed for 2 minutes in order to re-aerate the atmosphere in the jars. Following aeration, the mason jars were re-sealed and stored in a dark place.

Gas concentrations measured at each sampling time represented a cumulative N\(_2\)O production over the time elapsed since the last sampling period and were converted to kg N\(_2\)O-N ha\(^{-1}\), based on the actual packing density of the soil (1 g/cm\(^3\)) and depth of the sample (1 cm) for ease comparison to N application rates (in this case, \(\mu\)g N\(_2\)O-N g\(^{-1}\) soil = kg N ha\(^{-1}\) x 10). Total cumulative N\(_2\)O production was calculated by adding up the N\(_2\)O production from each sampling period.

**Statistical Analysis**
Statistical analyses were performed using the Proc MIXED procedure (SAS version 9.2; SAS Institute 2010, Cary, NC., USA) (Littell et al. 1998). The effect of fertilizer source, soil fertilization history and their interaction on cumulative N\textsubscript{2}O and CO\textsubscript{2} fluxes and other measured soil properties for the entire period of incubation were assessed by analysis of variance using a split plot design.

Prior to the statistical analysis, all the data were tested with respect to the assumptions of normality, independence and heteroscedasticity of residuals. Whenever necessary, log transformations were applied. Comparisons of least squares means were done using Tukey’s procedure and statistical significance was declared at P < 0.05.

For linear relationships between measured variables (post incubation NO\textsubscript{3}-N vs cumulative N\textsubscript{2}O-N production, and N\textsubscript{2}O-N production vs CO\textsubscript{2} – C production) orthogonal regression was used since both predictor and response variables contained measurement errors (Carroll and Ruppert 1996).

**RESULTS AND DISCUSSION**

**N\textsubscript{2}O Production in response to soil and fertilizer treatments**

Both soil history and current fertilizer treatments and their interaction were significant with respect to cumulative N\textsubscript{2}O production over the 7-week incubation (Table 3). As Fig. 1 shows, however, the majority of the soil history-by-fertilizer interactions were observed with the addition of urea and urea + S\textsubscript{0}. Following application of urea, soils with a long-term history of N fertilization (NPKS, NPK) had significantly greater cumulative N\textsubscript{2}O production than soils without a long-term history of N fertilization (Fig. 1). The greatest observed N\textsubscript{2}O production was
in the NPKS and NPK soils after addition of UR or UR + S\(^0\). Only in the Check soil did co-addition of S\(^0\) with urea result in N\(_2\)O production at the same level at the soils with a history of long-term N fertilization (Fig. 1).

With respect to fertilizer N source, our results were in agreement with several authors (Bergstrom et al. 2001; Bouwman et al. 2002; Gangon et al. 2011) who observed higher N\(_2\)O production following application of urea fertilizer. Similarly, in laboratory incubations, Pathak and Nedwell (2001) and Tenuta and Beauchamp (2003) observed N\(_2\)O production was greatest with urea, followed by NH\(_4^+\)-based fertilizers and least with NO\(_3^-\) based fertilizers, however, under anoxic conditions, NO\(_3^-\)-based fertilizers produced higher N\(_2\)O production. In our experiment, no difference was detected in cumulative N\(_2\)O production between NH\(_4^+\) based fertilizers and NO\(_3^-\) based fertilizers, and both types of fertilizers recorded the lowest production in all types of soils.

The underlying reason for the large differences in N\(_2\)O production from soils with different fertilization history following addition of urea is unclear. There were no strong correlations between pre-incubation soil properties (Table 2) and cumulative N\(_2\)O-N. Historical N additions to soils may increase soil N\(_2\)O production because: 1) long-term N inputs exceed harvest N removals resulting in accumulation of soil N over time as demonstrated in the meta-analysis of van Groenigen et al. (2010); 2) long-term urea inputs “prime” the soil microbial community to respond very quickly to current urea applications and this appears to increase soil N\(_2\)O production (Pearce 2016); and 3) long-term urea applications cause a shift in the microbial communities to respond very quickly to current urea applications. The former (1) suggests that soil N\(_2\)O production in response to current N applications is mostly a function of the long-term soil N balance. The latter, (2) and (3), suggests that soil N\(_2\)O production in response to current N
applications is a result of the long-term application of specific substrates for N cycling microorganisms and less a function of long-term soil N balance.

We did not observe a consistent association of high total soil N with high N\textsubscript{2}O production in the soils with different fertilization histories. For example, pre-incubation total N levels (an indicator of the long-term soil N balance), in the PKS and NPKS soils were identical (Table 2), but N\textsubscript{2}O production following application of urea was much higher in the NPKS soil (Fig. 1). Even though the PKS soil does not have a history of fertilizer N (urea) applications, it has a similar N balance to the NPKS treatment because of the biological N inputs of the alfalfa-brome phase of the rotation are greater than fertilizer N inputs during the cereal phases of this rotation. Therefore, in this case, soil N\textsubscript{2}O production in response to current urea applications appear to be more sensitive to past applications of urea rather than soil N balance.

Priming mechanisms with respect to N\textsubscript{2}O production in soil are not well investigated, but one possible mechanism is the presence of residual urease. Urease activities in soils receiving long-term applications of urea were higher than in non-fertilized treatments in semi-arid areas of India and China (Hu et al. 2014; Bhatt et al. 2016). Urease is remarkably stable and long-lived in soils, even following air-drying (Zantua and Bremner, 1977). Residual urease would facilitate urea hydrolysis following application which would create an ample supply of substrate for ammonia oxidizing and nitrifying bacteria which can produce N\textsubscript{2}O during their metabolism (Siciliano 2013).

The production of N\textsubscript{2}O in soil is influenced by the composition of the microbial communities which produce enzymes that affect the N transformations and N\textsubscript{2}O production such as urease, nitrite reductase, ammonia (NH\textsubscript{3}) mono-oxygenase and nitrous oxide reductase which reduces N\textsubscript{2}O to N\textsubscript{2} (Siciliano 2013). It may be possible that long-term urea applications in these soils
result in a shift in microbial community with a greater proportion of NH$_3$-oxidizing bacteria and archaebacteria - which can also produce urease (Koper et al. 2004) - that quickly decompose added urea N resulting in more N$_2$O (Böhme et al. 2005; Enwall et al. 2007; He et al. 2007; ). The effects of long-term fertilization on the composition of soil microbial communities and the possibility of historical fertilizer N application priming effects involved in nitrogen cycling warrants further investigation.

**N$_2$O production in relationship to CO$_2$ production and soil extractable NH$_4^+$, NO$_3^-$ and SO$_4^{2-}$**

Tables 2 and 4 summarize the pre- and post-incubation soil NH$_4^+$-N and NO$_3^-$-N levels. Almost all of the 100 kg N/ha added as NO$_3^-$ in the CN treatments (with and without S$^0$) was recovered as NO$_3^-$ (Table 4), and N$_2$O production from these treatments were not significantly different from the nill treatments for all soils with different fertilization histories (Fig. 1). It is also interesting to note that, in the AC treatments (with and without S$^0$), almost all of the 100 kg N/ha applied was recovered as NH$_4^+$-N (Table 4) and the average post-incubation soil NO$_3^-$-N levels were not different than average pre-incubation levels, likely because chloride significantly inhibits nitrifying bacteria (Souri 2010; Megda et al. 2014). Further, the N$_2$O production from the AC treatments were also not significantly different from the CN and nill treatments for all soils (Fig. 1). On the other hand, post-incubation levels of soil NH$_4^+$-N and NO$_3^-$-N were higher than pre-incubation levels in the urea treatments (with and without S$^0$) in all soils and it appears that most of the 100 kg N ha$^{-1}$ added as urea was recovered in these two fractions which is consistent with the conversion of urea to NH$_3$ and NO$_3$ via hydrolysis and nitrification. In Fig. 2, a significant linear relationship between post incubation NO$_3^-$-N concentration and N$_2$O flux for the UR and UR + S$^0$ fertilizer treatments is apparent, but the Check and PKS soils have a smaller
slope compared to NPKS and NPK soils. The only other dependent variable significantly influenced by the interaction between soil management history and fertilizer type was cumulative CO$_2$-C production (Fig. 3). The overall correlation between N$_2$O-N and CO$_2$-C production was $r = 0.64$ ($P < 0.0001$), but Fig. 3 shows soil-dependent linear relationships consistent with the soil-dependent linear relationships between post-incubation NO$_3^-$-N and N$_2$O-N. Soils without long-term N fertilization (Check and PKS) have a lower slope than soils with long-term N fertilization. Since the tested soils were incubated in aerobic atmospheres, apparently, the availability of oxygen (O$_2$) was sufficient for simultaneous respiration and net nitrification (at least in the urea treatments).

An estimate of apparent, cumulative, net nitrification is the difference between pre- and post-incubation soil NO$_3^-$-N minus any NO$_3^-$-N additions. Therefore, these results indicate that nitrification was likely only significantly active in the urea treatments. N$_2$O can be produced during nitrification via the hydroxylamine pathway and via nitrifier denitrification (Siciliano 2013) and the observed 10-fold increase in N$_2$O production from the urea treatments over control, AC and CN treatments (with and without S$^0$) may be a result of N$_2$O released from these two processes especially with the low WFPS conditions of this incubation (Kool et al. 2011). If anaerobic denitrification of soil NO$_3^-$ were a significant source of N$_2$O in the urea treatments, higher N$_2$O production from the CN treatments (with and without S$^0$), would be expected. Further, the hypothesis that the hydroxylamine pathway and nitrifier denitrification were sources of N$_2$O following addition of urea is consistent with the low N$_2$O production and low levels of post-incubation soil NO$_3^-$-N in the AC treatments – little apparent, cumulative, net nitrification was associated with low soil N$_2$O production.
Simultaneous nitrification and oxidation of elemental S were apparent in the UR and UR + S⁰ fertilizer treatments (Table 4) as indicated by the increased post-incubation NO₃⁻-N and SO₄²⁻-S in these treatments averaged over all soils. Based on the amount of recovered NO₃⁻-N and SO₄²⁻-S post-incubation from the UR + S⁰ treatment (Table 4), compared to the 100 kg N/ha and 20 kg S/ha application rates, a greater proportion of applied N was apparently nitrified (approximately 40%) compared to the proportion of applied S that was apparently oxidized (approximately 25%). This difference is potentially a result of a decreased rate of S oxidation compared to the rate of nitrification, O₂-limited conditions in the soil pore space, or a time lag in S oxidizing bacterial populations becoming active compared to nitrifying bacteria. Oxidation of elemental S did not appear to have any impact on the amount of apparent nitrification as indicated by similar post-incubation NO₃⁻-N levels in the UR and UR + S⁰ treatments. In contrast to these results, other authors have noted that S oxidation inhibits nitrification (Wainwright et al. 1986). If S-denitrification was an active process occurring in the urea + S⁰ treatments, it which would likely have been manifested as a decrease in post-incubation NO₃⁻ because NO₂⁻ and NO₃⁻ are used as electron acceptors in this process (Shao et al. 2010). On the other hand, post-incubation SO₄²⁻-S was greater in the UR + S⁰ treatment compared to the CN + S⁰ and AC + S⁰ treatments which suggests that S oxidation may have been enhanced when active in concert with nitrification.

CONCLUSIONS

The results reported here show that N₂O production following application of urea were significantly influenced by the long-term fertilization history - i.e., long-term soil treatments with a history of urea N fertilization (NPKS, NPK) had greater cumulative N₂O production following application of urea compared to soils without a history of N fertilization (Check, PKS). The elevated soil N₂O production from soils with a history of urea N application, did not appear to be
a response to greater long-term accumulation of total soil N in these treatments, but possibly a priming effect or shift in microbial communities induced by long-term urea applications.

Based on these results, we identify to the following knowledge gap and hypothesis:

1) If long-term urea applications do induce a priming effect with respect to soil N₂O production, the mechanism is not clear, but residual urease activity may be possible mechanism. The apparent, cumulative, net nitrification was comparable following application of urea to soils with and without a long-term history of urea application, but more N₂O was produced in soils with long-term urea applications. Because of the low WFPS (40%) conditions of this incubation, the priming effects likely influence N₂O production resulting from the hydroxyl-amine or nitrifier denitrification pathways (Kool et al. 2011). Greater residual urease activity in soils with long-term urea applications may favor N₂O production from the hydroxyl-amine pathway through a quick supply of ammonia to ammonia oxidizing bacteria.

2) Long-term fertilizer applications likely influence microbial community composition and ecology, but more investigations are required to elucidate these effects. Long-term urea applications could result in a greater abundance of urease-producing ammonia-oxidizing bacteria and archaeabacteria (Koper et al. 2004; He et al., 2007;) and other urease producing bacteria which may increase N₂O production via the hydroxyl-amine or nitrifier denitrification pathways following urea applications in well-aerated soil conditions (Kool et al. 2011).

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References


Table 1. Treatment descriptions in the Breton Classical Plots study, where the soil samples were taken

<table>
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<th>Treatments, 1930-1979 inclusive</th>
<th>Treatments, 1980 onward</th>
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<tr>
<td></td>
<td>kg ha(^{-1})</td>
<td>kg ha(^{-1})</td>
</tr>
<tr>
<td>5</td>
<td>Check 0 0 0 0</td>
<td>Check 0 0 0 0</td>
</tr>
<tr>
<td>3</td>
<td>NPKS 10 6 16 10</td>
<td>NPKS (^{b}) 22 46 0</td>
</tr>
<tr>
<td>7</td>
<td>NPKSL 11 6 16 9</td>
<td>NPK (^{b}) 22 46 0</td>
</tr>
<tr>
<td>8</td>
<td>P 0 9 0 0</td>
<td>PKS 0 22 46 0</td>
</tr>
</tbody>
</table>

\(^{a}\) “Plot” designation refers to the original design of the experiment (Dyck et al. 2012) and is associated with the physical location of the fertility treatments as the Breton Plots. It is included here for clarity and to be consistent with previous publications.

\(^{b}\) N (applied as urea) rate depends on the crop and its place in the rotation: wheat after forage (50 kg N ha\(^{-1}\)), oat or barley after wheat (75 kg N ha\(^{-1}\)), barley under seeded to hay: 50 kg N ha\(^{-1}\) and legume-grass forages: 0 kg N ha\(^{-1}\).

\(^{c}\) S is applied as elemental S at a rate of 5.5 kg S ha\(^{-1}\) from 1980 – 2007 and 20 kg S ha\(^{-1}\) from 2007 – present.

\(^{d}\) Rates represent rates of the nutrient element (P or K) rather than P\(_2\)O\(_5\) and K\(_2\)O convention. P is applied as triple super phosphate (0-46-0) and K is applied as muriate of potash (0-0-62).
Table 2. Selected soil properties for the 0 to 10 cm depth for each soil treatments of the sampling site (Breton Classical Plots) in the current study

<table>
<thead>
<tr>
<th>Soil</th>
<th>TOC (kg C/ha)</th>
<th>TN (kg N/ha)</th>
<th>C/N ratio</th>
<th>LFC (kg C/ha)</th>
<th>LFN (kg N/ha)</th>
<th>NH$_4^+$-N (kg N/ha)</th>
<th>NO$_3^-$-N (kg N/ha)</th>
<th>TS (kg S/ha)</th>
<th>SO$_4^{2-}$-S (kg S/ha)</th>
<th>pH</th>
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<tbody>
<tr>
<td>Control</td>
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<td>201</td>
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<td>98</td>
<td>5.9</td>
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<td>0.58</td>
<td>37.0</td>
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</tr>
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</table>

Note: TOC, TN: total C, total N, combustion; LFC, LFN: light-fraction C, N, physical fractionation; NH$_4^+$-N, NO$_3^-$-N, KCl extractable, TS: total S, nitric acid digest; pH, 5:1 CaCl$_2$.

Unit conversion: µg g$^{-1}$ = kg ha$^{-1}$ x 10
**Table 3.** Summary of the P value of the analysis of variance (ANOVA) comparing the effect of soil, fertilizer, and soil x fertilizer interaction on cumulative NH$_4^+$-N, NO$_3^-$-N, SO$_4^{2-}$-S content, and N$_2$O-N and CO$_2$-C production during the seven-week incubation period

<table>
<thead>
<tr>
<th></th>
<th>NH$_4^+$-N</th>
<th>NO$_3^-$-N</th>
<th>SO$_4^{2-}$-S</th>
<th>cumulative N$_2$O-N production</th>
<th>cumulative CO$_2$-C production</th>
</tr>
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<tbody>
<tr>
<td><strong>P-value</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Soil</td>
<td>0.0079</td>
<td>0.3222</td>
<td>0.7206</td>
<td>0.0078</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Fertilizer</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Soil x Fertilizer</td>
<td>0.5705</td>
<td>0.6706</td>
<td>0.9314</td>
<td>&lt; 0.0004</td>
<td>0.0499</td>
</tr>
</tbody>
</table>
**Table 4.** Effect of fertilizer treatments (Mean (n=6) on soil inorganic N (NH$_4^+$-N, NO$_3^-$-N), and SO$_4^{2-}$-S (kg ha$^{-1}$) in incubated soils over seven weeks of incubation. Associated standard errors are shown in parenthesis.

<table>
<thead>
<tr>
<th>Fertilizer</th>
<th>NH$_4^+$-N</th>
<th>NO$_3^-$-N</th>
<th>SO$_4^{2-}$-S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>0.77 (0.05) d</td>
<td>10.6 (0.30) c</td>
<td>1.2 (0.24) c</td>
</tr>
<tr>
<td>UR</td>
<td>42.2 (2.54) b</td>
<td>52.3 (3.39) b</td>
<td>1.0 (0.14) c</td>
</tr>
<tr>
<td>CN</td>
<td>9.5 (0.93) c</td>
<td>104.3 (2.93) a</td>
<td>0.4 (0.11) c</td>
</tr>
<tr>
<td>AC</td>
<td>103.6 (2.54) a</td>
<td>3.2 (0.97) c</td>
<td>0.8 (0.15) c</td>
</tr>
<tr>
<td>UR+S$^0$</td>
<td>45.8 (2.46) b</td>
<td>45.6 (3.47) b</td>
<td>6.2 (1.12) a</td>
</tr>
<tr>
<td>CN+S$^0$</td>
<td>8.9 (0.96) c</td>
<td>96.2 (3.05) a</td>
<td>3.7 (0.82) b</td>
</tr>
<tr>
<td>AC+S$^0$</td>
<td>106.9 (3.2) a</td>
<td>2.2 (0.69) c</td>
<td>4.2 (0.98) b</td>
</tr>
</tbody>
</table>

**Note:** Values in the same column sharing the same letters are not significantly different at (P < 0.05) probability level.

Nil: unfertilized, UR: urea, AC: ammonium chloride, CN: calcium nitrate, UR+S$^0$: urea plus elemental sulfur, AC + S$^0$: ammonium chloride plus elemental sulfur, CN + S$^0$: calcium nitrate plus elemental sulfur. S$^0$.

Unit conversion: µg g$^{-1}$ = kg ha$^{-1}$ x 10
Fig. 1. Soil and fertilizer treatments interaction effect on cumulative $\text{N}_2\text{O}$-N production (kg N/ha). The height of the bars represents the mean, cumulative N$_2$O-N produced in each soil-fertilizer treatment and error bars represent one standard error. Shared letters indicate means are not significantly different (P < 0.05). Unit conversion: $\mu$g N g$^{-1}$ soil = kg N ha$^{-1}$ x 10.
Fig. 2. Relationship between cumulative N$_2$O-N production and post incubation NO$_3$-N over a seven-week of incubation period from four soils with different fertilization history - soil without fertilizer application (Control), soil with long-term application of NPKS, soil with long-term application of NPK, and soil with long-term application of PKS. Symbols represent means of variables and error bars represent 1 standard error. Colored lines on the graph correspond to the colors of the symbols and represent the orthogonal regression between post-incubation NO$_3$-N and cumulative N$_2$O-N production, excluding the measurements from CN and CN + S$^0$ treatments. The slopes of the regression lines are: 0.012, 0.023, 0.026 and 0.009 log(kg N$_2$O-N)/kg NO$_3$-N for the Control, NPKS, NPK and PKS soils respectively. The intercepts of the regression lines are -1.137, -1.183, -1.139, -1.219 for the Control, NPKS, NPK and PKS soils respectively.
respectively. All slope and intercept estimates were highly significant (P < 0.001). Unit conversion: \( \mu g \) N g\(^{-1}\) soil = kg N ha\(^{-1}\) x 10

**Fig. 3.** Relationship between N\(_2\)O-N production and CO\(_2\)-C production over a seven-week incubation period from four soils with different fertilization history - soil without fertilizer application (Control), soil with long-term application of NPKS, soil with long-term application of NPK, and soil with long-term application of PKS. Symbols represent means of variables and error bars represent 1 standard error. Colored lines on the graph correspond to the colors of the symbols and represent the orthogonal regression between cumulative CO\(_2\)-C and cumulative N\(_2\)O-N production. The slopes of the regression lines are: 0.027, 0.053, 0.073 and 0.031 log(kg N\(_2\)O-N)/kg CO\(_2\)-C for the Control, NPKS, NPK and PKS soils respectively. The intercepts of the regression lines are -1.929, -2.385, -3.626, -2.255 for the Control, NPKS, NPK and PKS soils respectively.
respectively. All slope and intercept estimates were highly significant (P < 0.001). Unit conversion: \( \mu g \, g^{-1} \, soil = kg \, ha^{-1} \times 10 \)