4R NUTRIENT STEWARDSHIP:

Nutrient Supply and GHG Emissions from a S-deficient Soil as a Function of Fertilization History

CANADIAN FERTILIZER INSTITUTE
INSTITUT CANADIEN DES ENGRAIS

4R nutrient stewardship

Project Final Report

Project Title:

Nutrient Supply and GHG Emissions from a S-deficient Soil as a Function of Fertilization History

The University of Alberta Breton Plots

Submitted to

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Executive Summary

It has been reported that "balanced" fertilization (that is balancing application of other essential nutrients like S in addition to N, P, and K) can significantly increase crop apparent recovery of applied N (Flynn, 2009). The hypothesis tested in this research was that N_2O emissions would be lower from soils with a history of balanced fertilization (i.e., NPKS) than soils with a history of unbalanced fertilization (NPK, PKS, and no fertilizer application).

A laboratory incubation experiment and field gas flux measurements were conducted in order to assess the nutrient and N_2O production response of selected treatments of the University of Alberta Breton Classical Plots. The Breton Classical Plots were established in 1930 on a S-deficient Gray Luvisolic soil near Breton, AB and have had consistent fertility treatments imposed on two rotations (5- and 2-year) since 1980. Our results showed that N_2O emissions were 5 – 20% lower when the soil had a history of balanced fertilization (NPKS) compared to a history of unbalanced fertilization.

The reduced N_2O emissions observed on balanced fertilization treatments are hypothesized to be attributable to: 1) enhanced N recovery by crops under balanced fertilization; and 2) an interaction between S and N cycling in the soil whereby nitrite (a precursor to N_2O production during nitrification) is used as an electron acceptor and converted to N_2 by S-oxidizing bacteria during elemental S oxidation. This N-S cycling interaction was not directly observed in this research, but is a hypothesis that will be tested in future research. If the hypothesized N-S interaction is operational in agricultural soils, banding of urea and elemental S together may reduce N_2O production from nitrifier denitrification in agricultural soils.

A summary of the key findings of this research with respect to the 4R/NERP frameworks is presented below.

4R/NERP Principle	Application of results from this research
Right Product	1) In soils where nitrifier denitrification is the main N_2O production process, the banding of elemental S with ammonium-based fertilizers may help to reduce nitrous oxide emissions because S-oxidizing bacteria can use nitrite as an electron acceptor and the end result is N_2 instead of N_2O. This process was not directly observed in this research, but has been observed in other systems (wastewater treatment systems and estuarine environments) and is a hypothesis generated by the observations from this research that soils with long-term NPKS fertilization had 5 – 20% lower N_2O-N emissions than soils with long-term NPK or PKS fertilization. This result also demonstrates the importance of long-term balanced fertilization on residual soil nutrient levels. If this hypothesized N-S interaction is active, soil fertilizer N is still lost to the environment in a more benign form (i.e., N fertilization use efficiency is not likely to be increased).

	2) This research observed that soils with low pH (acidic) had significantly greater N_2O emissions than soils with higher pH. At the Breton plots, cumulative growing season emissions increased by 5 kg N_2O-N/ha for every 1.0 decrease in pH. Liming is recommended to maintain soil pH above 6.0. 3) Ammonia-based fertilizers are recommended because they are less susceptible to leaching. However, this research showed that in soils where the dominant N_2O production process is nitrifier denitrification, ammonia-based fertilizers may not be the right product because oxidation of ammonium produces nitrite which is the precursor of nitrous oxide production in this process. The use of nitrification inhibitors might help to significantly N_2O emissions where nitrifier denitrification is the main N_2O production process.
Right Rate	This research showed that high levels of residual N from previous years can significantly increase nitrous oxide fluxes at Breton. This was shown by the observation that N_2O fluxes in wheat following two years of alfalfa-brome in the WOBHH rotation were up to 3X greater than the wheat-fallow rotation despite a much lower N fertilizer application rate (50 kg N/ha for WOBHH; 90 kg N/ha for WF). Even though N fertilizer is not applied in the hay phase of this rotation, alfalfa contributes to increased N levels in the soil through N-fixation and through N-rich residues which are ploughed under prior to seeding annual crops. Coupled with applications of N fertilizer to subsequent annual crops, it would appear that this rotation has resulted in the accumulation of excess residual N over the long-term. In order to avoid excess residual N, fertilizer N recommendations appropriate for the crops in the rotation based soil tests are recommended.
Right Place/Right time	This research observed that significant losses of N fertilizer to N_2O can occur following application if fertilizer is broadcast and incorporated especially in acidic soils. The method for fertilizer applications at the Breton Plots is to broadcast and incorporate with tillage prior to spring seeding. This application of N fertilizer to moist soil followed by tillage, increases soil oxygen levels, soil temperature and microbial activity. As a result, the urea N fertilizer is quickly transformed to ammonium which is then oxidized to nitrite which is susceptible to nitrifier denitrification. Nitrous oxide losses were greater in soils with low pH. Banding N fertilizer in the spring or late fall slows down the transformation of N fertilizers because the soil is not disturbed to the same extent during banding compared to incorporation with tillage. Banding fertilizers decreased N_2O emissions.

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Research Project Summary

Title: Nutrient Supply and GHG Emissions from a S-deficient Soil as a Function of Fertilization History

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Contents

1. Introduction

Agriculture is considered to be a major source of greenhouse gas (GHG) emissions (CO_2, N_2O and CH_4), which drive global warming potential (Snyder et al., 2009, Linquist et al., 2012). One-fifth of the annual increase in anthropogenic greenhouse warming originates from agriculture (Intergovernmental Panel, 2007). Agriculture is estimated to produce about 50% and 70% of anthropogenic CH_4 and N_2O emissions (IPCC, 2007). Unlike N_2O and CH_4, however, CO_2 is fixed by crop plants through photosynthesis and is largely offset by net productivity, (Snyder et al., 2009, Linquist et al., 2012), and contributes less than 1% of the global warming potential (GWP) of agriculture (Smith et al., 2007). In Canada, agriculture contributes less than 8% of the GHGs emissions inventory (Environment Canada, 2007).

The global warming potential of N_2O is 310 times greater than CO_2 and has an 120-year atmospheric lifetime which makes it an important trace gas next to CO_2 and methane (CH_4) (Braker and Conrad, 2011). Currently, the global N_2O budget is imbalanced, as the total sink strength of about 14 Tg N_2O y^{-1} is smaller than the total source strength of about 17.7 Tg N_2O y^{-1} (IPCC, 2007).

Although there are several biological /microbial processes that are involved in N_2O production such as dissimilatory nitrate reduction to ammonia, nitrate assimilation, chemodenitrification and codenitification (Batjes and Bridges, 1992; Bremner, 1997; Wlodarczyk et al., 2004; Braker and Conrad, 2011), N_2O in most soils is produced during nitrification (NH_4^+ to NO_3^-) and to a lesser extent anaerobic denitrification (Paustian et al., 2006; Subbarao et al., 2006, Flynn, 2009, Braker and Conrad, 2011). Even though autotrophic nitrification is considered commonly to be the main source for N2O in most soils, heterotrophic nitrification also contributes to N_2O production, particularly in acidic soils (Batjes and Bridges, 1992; Bremner, 1997; Braker and Conrad, 2011). However, the nitrification rate is usually significantly higher in autotrophic than in heterotrophic nitrification (Braker and Conrad, 2011). Autotrophic nitrification is a two-step process summarized in the following two reactions (Batjes and Bridges, 1992):

$$NH_4^+ + 3/2\ O_2 \rightarrow NO_2^- + 2H^+ + H_2O + E \quad [1a]$$
$$NO_2^- + 1/2\, O_2 \rightarrow NO_3^- + E \quad [1b]$$

According to Groffman (1991) and Braker and Conrad (2011), N_2O production is possible during both steps.

During the first step, ammonium (NH_4^+) and/or ammonia (NH_3) are oxidized to nitrite (NO_2^-) and ultimately to nitrate (NO_3^-), by chemolithoautotrophic ammonia and nitrite oxidizers and mainly accomplished by chemolithoautotrophic bacteria in the genus Nitrosomonas, and N_2O is produced by the chemical decomposition of hydroxylamine (NH_2OH) during this step (Batjes and Bridges, 1992; Braker and Conrad, 2011).

The second step (nitrite to nitrate) is performed by the genus nitrobacter. Nitrifier denitrification occurs during this step when autotrophic ammonia-oxidizing bacteria use NO_2^- as an alternative electron acceptor when O_2 is limiting and produce N_2O through subsequent reduction of NO to N_2O.

Even if there is evidence that nitrification can occur under anaerobic conditions (Wlodarczyk et al., 2004), it mainly occurs under aerobic conditions (Bremer, 1997; Braker and Conrad, 2011). Under an anaerobic conditions, where oxygen is absent, and water, nitrate and decomposable organic compounds are present, aerobic bacteria may use nitrate (NO_3^-), nitrite (NO_2^-) and nitrous oxide (N_2O) as alternative electron acceptors and return molecular N_2 to the atmosphere through the process of denitrification (Batjes and Bridges, 1992; Braker and Conrad, 2011). The process of denitrification can be presented as follows (Batjes and Bridges, 1992; Bremner, 1997):

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad [2]$$

Aerobic heterotrophs such as (Pseudomonas, Alcaligenes and Azospirillum) and autotrophic organisms such as (Paracoccus denitrificans, Rhodopseudomonas sphaeroides and Thiobacillus denitrificans) could be involved in the pathway of denitrification (Batjes and Bridges, 1992).

Although dentrification has been commonly accepted to occur under anaerobic and anoxic conditions, there have been some reports of aerobic denitrification (Robert and Kuenen, 1984; Lampe and Zhang, 1996; Beller et al., 2006; Cardoso et al., 2006).

Among several environmental, physical and chemical factors that influence nitrification in the soil, texture and structure, moisture status, aeration (O_2 and CO_2 levels), soil matrix, temperature , pH , electrical conductivity (EC), organic matter , C/N ratio and cation exchange capacity play a major role in nitrification (Subbarao et al., 2006; Sahrawat, 2008; Braker and Conrad, 2011). Likewise, denitrification can be influenced by the aeration/moisture status of the soil, the nature and amount of organic matter available as energy source to denitrifiers, soil pH, soil texture, the soil nitrate concentration and soil temperature (Bremner, 1997; Wlodarczyk et al., 2004).

Although N_2O production and emissions from soil vary due to various factors, which are described above, there is a rough proportionality between N entering to the soil from anthropogenic inputs (i.e. fertilizer, manure, plant legumes) and the amount lost as N_2O (Bowman , 2002). In line with this, Flynn (2009) also pointed out that, although the exact relationship between nitrogen inputs and N_2O emissions is debatable, when added N fertilizer exceeded plant uptake capacity, N_2O emissions increase dramatically.

Emissions of greenhouse gases from agricultural soils could be mitigated by best fertilizer management practices (Snyder et al., 2009; Linquist et al., 2012). These practices are based on the principles of the 4-R's: " right source, at the right rate, at the right time, and with the right placement in order to deliver the economic, social and environmental benefit expected by the society (Snyder et al., 2009; Bruulsema et al., 2009; IFA, 2009). For example, Although N mineralization from organic matter during the growing season contributes to a considerable proportion of the soil N available to plants, care should be taken to reduce environmental concerns and attain economical fertilization (Flynn, 2009; Snyder et al; 2009; Linquist,2012).

Soil nutrient management research in agricultural systems has generally taken a "one nutrient at a time" approach with limited attention to the interaction between nutrients (Havlin et al., 1999). In this context, Flynn (2009) reported that "balanced" fertilization (that is balancing application of other essential nutrients like S in addition to N, P, and K) can significantly increase crop apparent recovery of applied N. Improved crop recovery can reduce N fertilizer-associated greenhouse gas emissions and global warming potential by reducing NH_3 loss and NO_3 -N leaching (Snyder et al., 2009) and likely also N_2O emissions. There is evidence to suggest that macronutrient transformation processes in soil affect one another. Previous research by Cardoso et al. (2006), Beller et al. (2006) and Zhou et al. (2011) indicated that chemolithoautotrophic dentirifiers (anaerobic facultative bacteria) such as Thiobacillus denitrificans and Thiomicrospira denitrificans oxidize reduced inorganic sulfur compounds such as sulfide (S^{2-}).elemental sulfur (S^0), thiosulfate ($S_2O_3^{2-}$), or sulfate (SO_3^{2-}), or sulfite (SO_3^{2-}) by using nitrate/nitrite an electron acceptors resulting in the production of N_2 gas. Sulfur oxidation by these organisms can occur both under aerobic and under anoxic conditions (Beller et al. (2006; Cardoso et al., 2006). These organisms use inorganic carbon compounds (e.g., CO_2, HCO_3^-) as their carbon source and the oxidation reactions of inorganic of inorganic sulfur compounds as energy source (Lampe and Zhang, 1996; Zhou et al., 2011).

Sulfur utilizing autotrophic denitrifiers can be found in both soil and water habitats, and are believed to play an important role in mineral cycling by linking nitrogen and sulfur cycles (Korom, 1992). In line with this, Sue et al. (2012) showed that autotrophic denitrification catalyses the oxidation of sulfur coupled with the reduction of nitrate to nitrite, reporting an interaction between N and S, which was reflected in increasing of the sulfate during the reduction of nitrate and nitrite, while nitrate was used by sulfur oxidizing bacteria as a terminal acceptor. Other studies (Lampe and Zhang, 1996; Cardoso et al., 2006; Zhou et al., 2011) also confirmed that during autotrophic denitrification sulfur was used as an electron donor and nitrate was reduced to nitrite, and subsequently to nitrogen gas. Similarly, although strong inhibition was observed at high nitrate concentrations, nitrite reduction was also coupled with sulfur oxidation. Therefore, as all these studies demonstrated, sulfur-driven autotrophic denitrification, and N-S interactions can influence N2O emissions.

Although there is approximately 4 million ha S-deficient soils in the cultivated areas of the Canadian prairie Provinces, and additional 8 million ha estimated to be potentially deficient (Solberg et al., 2005), much of the research to date has focused on the effect of N fertilization on N_2O emission and /or carbon sequestration. To our knowledge, although few studies have been conducted on the combined effect of S and N –fertilization on carbon sequestration (Nyborg et al., 1999; Malhi et al., 2010; Malhi et al., 2012), there is currently limited published reports on the effect of combined application of Nitrogen (N) and sulfur (S) fertilizers in soils in terms of nutrient supply (N and S) and GHG (N_2O) emission with respect to the long-term management history (fertilization and rotation), particularly in sulfur-deficient soils of western Canada.

Therefore, the objective of this research is to investigate the interaction of both S and N fertilization with respect to fertilization history on an S-deficient soil on N_2O emission and nutrient supply (N and S) through field and laboratory experiments. Furthermore, a better understanding of the N and S fertilizer interactions with respect to nutrient supply/ availability and N_2O emissions in sulfur deficient soils will help to contribute to knowledge needed to adopt the good fertilizer management practices that mitigate inefficient use of fertilizers, deleterious environmental effects and mitigation of N_2O emission associated with fertilizers.

We hypothesised that the interaction between N and S transformation processes in the tested soils would decrease the N_2O emissions.

Therefore, the objectives of this study were (a) Quantify potential nutrient supply and GHGs production potential as a function of fertilization history, and added fertilizer N and S; and (b) to investigate and quantify N and S fertilizers interaction with respect to soil nutrient (plant available N and S) availability and GHG (N_2O) emission on sulfur-deficient soils collected at the University of Alberta Breton Plots, with different long-term fertilization histories.

2. Materials and Methods

2.1. The University of Alberta Breton Plots

Field GHG fluxes and the soils for the incubation experiment were collected from the University of Alberta Breton Classical Plots. The Breton Classical Plots, established in 1930, represent the longest on-going experiment at the University of Alberta Breton Plots site (Dyck et al., 2012). They were established to address some of the challenges faced by early farmers on Gray Luvisolic Soils in northwestern Alberta. The original treatments of the Classical Plots were intended to test which nutrients were deficient in these gray soils. Another objective was to gain better understanding of which rotations would perform better on these soils. A schematic layout of the Classical Plots is presented in Figure 1 and treatment descriptions are presented in Table 1. The 11 treatments described in Table 1 are applied to two rotations: a five-year wheat-oats-barley-hay-hay (WOBHH) rotation and a 2-year wheat-fallow (WF) rotation. The five-year rotation is implemented on the north-south oriented "series" A, B, C, D and F (Fig. 1) and the wheat-fallow rotation is implemented on series E. Fertility treatments are oriented east-west across the rotations series.

The rotations and fertility treatment descriptions come with a few caveats and management notes:

1) The two rotations were originally a four-year rotation (3 years of cereals and 1 year of legumes) and continuous wheat between the years of 1930 – 1938/1939. In 1938, the continuous wheat rotation was changed to the wheat-fallow system that remains to this day. In 1939, the four-year rotation was converted to the five-year WOBHH rotation that remains to this day. The forage crops have varied over the years, but have always included a legume. Since 1967 the hay crops have been alfalfa-brome mixtures.
2) Starting in 1980, the fertility treatments were updated to reflect higher nutrient application rates commonly used for modern cereal varieties and to better test the crop responses to individual nutrients.
3) Fertilizer application and placement varied during the period of the original treatments (1930 -1979). Fertilizers were broadcast initially. From 1946-1964, fertilizers were applied every other year. Starting in 1964, annual applications were resumed and phosphorus was seed-placed while N, S and P are broadcast and incorporated. For the manure treatment, manure was applied every five years to achieve the annual rates listed in Table 1.
4) Lime was applied to plots 6 and 7 several times between 1930 and 1948 adding up to a total of 6.6 t ha^{-1}. Since 1972, lime has been added to the east half of all plots all whenever the soil pH measured was less than 6.0. Currently, lime rates are calculated so that soil pH can be restored to 6.5. Using these guidelines, the resulting frequency of lime application to the east half of the plots is about every 5 years or so.
5) When binders were still in use, harvest involved the removal of straw from the Plots and this practice continued to 1980. Since then, straw has been retained on the plots with the use of combine harvesters. Tillage and weed control has varied over the years. Herbicides have been used on the Plots since 1964.

Grain, straw, hay and silage yields are available for almost all of the growing seasons since 1930 (barley was cut for silage a few times since the start of the Classical Plots). While there have been crop and management changes over time, great efforts have been made to quantify additions of nutrients to the soil, and removals of nutrients from the soil. For example, from 1930 – 1939, legumes were ploughed down as green manure rather than cut for hay. Since 2000, barley underseeded with alfalfa and brome has been removed as silage rather than harvested just for barley grain. An electronic database is a work in progress to fully quantify these additions and removals. Currently, detailed soil sampling of the Plots is carried out every five years.

Results from the Classical Plots (Wyatt and Newton, 1932; McAllister, 1934; Wyatt, 1936) and Breton soil in the greenhouse (Wyatt et al., 1930) showed strong response by clover and wheat to added nitrogen (N) and Phosphorus (P) fertilizers. As a result of the early treatment design, however, N and sulphur (S) were applied together as ammonium sulfate and the individual contribution of S was overlooked. The deduction of the response of crops to S came later (Newton, 1936; Newton and Ignatieff, 1939; Newton, 1952; Nyborg and Bentley, 1971; Bentley et al., 1971). Thus, it was not possible to truly isolate the effects of S on crop production and soil characteristics until 1980 when treatment 7 was changed from NPKS plus lime to NPK[-S]. Currently fertilizer N, P, K and S products in use are urea (46-0-0-0), super triple phosphate (0-45-0-0), potash (0-0-60-0) and elemental sulfur (0-0-0-90)

Table 1: Treatment Descriptions in the Breton Classical Plot Study.

	treatments 1930 – 1979 inclusive[+]					treatments, 1980 onward				
		kg ha^{-1}					kg ha^{-1}			
Plot	Treatment	N	P	K	S	Treatment	N	P	K	S
1	Control	0	0	0	0	Control	0	0	0	0
2	Manure(M)	76	42	91	20	Manure	#			
3	NPKS	10	6	16	10	NPKS	*	22	46	5.5
4	NS	11	0	0	11	NSK(-P)	*	0	46	5.5
5	Control	0	0	0	0	Control	0	0	0	0
6	Lime(L)	0	0	0	0	Lime	0	0	0	0
7	NPKSL‡	11	6	16	9	NPK(-S)	*	22	46	0
8	P	0	9	0	0	PKS(-N)	0	22	46	5.5
9	MNPS	86	48	91	28	NPKS†	*	22	0	5.5
10	NPS	10	6	0	8	NPS(-K)	*	22	0	5.5
11	Control	0	0	0	0	Control	0	0	0	0

[+] fertilizer application rates of the original treatments varied (see text in article); manure was applied every 5 years to achieve the annual rates listed

*N is currently applied as urea and rates depend on the crop and its place in the rotation:

- wheat on fallow: 90 kg N ha^{-1}
- wheat after forage: 50 kg N ha^{-1}
- oats or barley after wheat: 75 kg N ha^{-1}
- barley underseeded to hay: 50 kg N ha^{-1}
- legume-grass forages: 0 kg N ha^{-1}

N application via manure depends on the rotation.

- wheat-fallow: 90 kg N ha^{-1} during cropped years
- cereal crops in WOBHH rotation: 175 kg N ha^{-1} every 5 years applied in two equal applications

† subsoiled in 1980

‡ lime plus P (LP) from 1930 – 1963. NPKSL from 1964-1979. This has a major effect on yields and the added S had residual effects for years after modification in 1980 to NPK (-S).

N↑

F	E	D	C	B	A	Series
WOBHH	W-F	WOBHH	WOBHH	WOBHH	WOBHH	Rotation
Control	Control	Control	Control	Control	Control	1
Manure (M)	Manure (M)	Manure (M)	Manure (M)	Manure (M)	Manure (M)	2
NPKS	NPKS	NPKS	NPKS	NPKS	NPKS	3
NS [NSK(-P)]	NS [NSK(-P)]	NS [NSK(-P)]	NS [NSK(-P)]	NS [NSK(-P)]	NS [NSK(-P)]	4
Control	Control	Control	Control	Control	Control	5
Lime(L)	Lime(L)	Lime(L)	Lime(L)	Lime(L)	Lime(L)	6
NP / NPKSL [NPK(-S)]	NP / NPKSL [NPK(-S)]	NP / NPKSL [NPK(-S)]	NP / NPKSL [NPK(-S)]	NP / NPKSL [NPK(-S)]	NP / NPKSL [NPK(-S)]	7
P [PKS(-N)]	P [PKS(-N)]	P [PKS(-N)]	P [PKS(-N)]	P [PKS(-N)]	P [PKS(-N)]	8
MNPS[NPKS]	MNPS[NPKS]	MNPS[NPKS]	MNPS[NPKS]	MNPS[NPKS]	MNPS[NPKS]	9
NPS [NPS(-K)]	NPS [NPS(-K)]	NPS [NPS(-K)]	NPS [NPS(-K)]	NPS [NPS(-K)]	NPS [NPS(-K)]	10
Control	Control	Control	Control	Control	Control	11

Plot/Treatment

Figure 1: Layout of the Breton Classical Plot Study. See Table 1 for a complete description of the treatments. Treatments in square

2.2. Soil Incubation Experiment.

The incubation experiment was a split-plot design with three replicates. Main plot treatments consisted of fertilization history of the soil. In the spring of 2012, soil was collected from the 0-15 cm surface layer of the Control, NPKS, NPK and PKS treatments of the wheat-oats-barley-hay-hay rotation of the Breton Classical Plots and was subsequently dried and ground. Therefore the main plot treatments will be named in accordance to the Breton plot treatment name: 1) control (no fertilizer); 2) NPKS; 3) NPK; and 4) PKS. Some basic soil properties of the soils taken from series C are presented in Table 2.

Table 2: Selected characteristics of surface soils (0-15 cm) of selected treatments of series C at the Breton Classical plots (unpublished data; samples taken in 2008)

Soil	TOC ($Mg\ ha^{-1}$)	Total N ($Mg\ ha^{-1}$)	C:N ratio	Total S (kg/ha)	pH
Control	32.5	3.1	10.4	16	6.8
NPKS	38.2	3.7	10.5	340	6.6
NPK(-S)	34.7	2.9	11.9	56	6.8
PKS(-N)	34.9	3.2	10.8	234[z]	6.6

Subplot treatments were: 1) no added fertilizer (NIL); 2) N fertilizer added (urea and ammonium phosphate → NP [100-80-0-0]); 3) Shell Thiogro S fertilizer added (ammonium phosphate sulfate plus elemental S; 12-44-0-11; 2 parts elemental S, 1 part sulfate-S) → Shell Thiogro S [22-80-0-20]; 4) conventional (non-Shell Thiogro) S fertilizer added (ammonium phosphate sulfate + elemental S) → Conv. S [22-80-0-20]; 5) conventional N & S fertilizer (all S supplied as sulfate; urea + ammonium sulfate) → NPS [100-80-0-20]; and 6) conventional N fertilizer plus Shell Thiogro S fertilizer → NP Shell Thiogro S [100-80-0-20] and 7) conventional N fertilizer plus Shell Thiogro S fertilizer plus biochar (5% by weight) → NP Shell Thiogro S Biochar [100-80-0-20].

In the lab, the ground, dry soil was wetted to 20% water content (field capacity) by weight and packed into 500 ml mason jars to a consistent bulk density of 1.0 g cm^{-3}, with 100 ml of nutrient solutions such that the target rates of nutrients were acheived. Target rates for nutrients were: 100 kg N ha^{-1}, 80 kg ha^{-1} P_2O_5 and 20 kg S ha^{-1}. Because of the Thiogro fertilizer formulation, additional phosphorus is added to all other treatments with S and an equivalent rate of 80 kg ha^{-1} P_2O_5 was be added to the NP treatment.

The soil microcosms were incubated aerobically for 12 weeks at room temperature. Nutrient supply (ammonium, nitrate and sulfate) was assessed using plant root simulator (PRS™) probes from Western Ag Innovations. PRS probes were installed in every soil column and replaced biweekly throughout the experiment. Water content was maintained at 20% by adding more water when required on a weekly basis.

Soil carbon dioxide (CO_2) and N_2O fluxes were measured weekly. The soil GHG emissions were measured using the nonsteady-state chamber method (Rochette and Bertrand, 2008) and a Innova photaccoustic infrared portable gas analyzer.

Total nutrient supply [total adsorbed N(NO3 + NH4), NO3, NH4, P, K, S and Al] and GHG emissions (CO_2 and N_2O) measured over the entire incubation period were analyzed with an ANOVA algorithm appropriate to the experimental design. When the ANOVA F-test of main plot, subplot or interactions was significant, means were compared using the least significant difference (LSD) The total N_2O efflux and total adsorbed available P data were log transformed prior to statistical analysis because they were log-normally distributed.

Field Gas Flux Measurements

Field CO_2 and N_2O fluxes were measured weekly during the 2013 growing season from plot numbers: 2 [Manure], 3 [NPKS], 5 [Control], 7 [NPK(-S)], 8 [PKS(-N)] and 11 [Control] on Series D (WOBHH rotaion – wheat phase) and E (wheat-fallow) rotation (Table 3). A summary of selected soil properties is presented in Table 4. Gas fluxes were taken from both east (regular lime applications) and west (no lime added) halves of the plots in Series D (WOBHH rotaion – wheat phase) and E (wheat on west half, fallow on east half). Like the laboratory incubation experiment, The soil GHG emissions were measured using the nonsteady-state chamber method (Rochette and Bertrand, 2008) and a Innova photaccoustic infrared portable gas analyzer.

The wheat phase of the WOBHH rotation was chosen because the soil samples collected for the laboratory incubation were following the wheat phase and because we could compare it to the cropped half of the wheat-fallow rotation. Because the WOBHH rotation at the classical plots is not fully phased, the treatments are not replicated for the wheat phase in any one year, making statistical analysis difficult. This limitation can be overcome by repeated measurements on the wheat phase over many growing seasons and it is our intent to do this, however, for this report, only 1 growing season of data is available.

Table 3: Diagram of plots where gas flux measurements were taken in 2013 (shaded gray). Plot/treatment details are given in Table 1.

E		D		Series	
W-F		WOBHH-Wheat Phase		Rotation	
				1	Plot/Treatment
F	W	NL	L	2	
F	W	NL	L	3	
				4	
F	W	NL	L	5	
				6	
F	W		L	7	
F	W	NL	L	8	
				9	
				10	
F	W	NL	L	11	

Table 4: Selected average characteristics of surface soils (0-15 cm) of selected treatments of series D at the Breton Classical plots (unpublished data; samples taken in 2008)

Soil/Rotation	Total C ($Mg\ ha^{-1}$)	Total N ($Mg\ ha^{-1}$)	C:N ratio	Total S (kg/ha)	pH
WOBHH					
Control					
Lime	37.6	3.60	10.6	277	7.0
no lime	37.4	3.58	10.2	198	6.3
Manure					
lime	48.4	4.98	9.7	727	6.8
no lime	46.8	4.88	9.6	692	6.3
NPKS					
Lime	38.9	3.65	10.7	523	6.5
no lime	38.4	3.86	10.0	619	5.2
NPK(-S)					
Lime	38.1	3.44	10.2	NA	7.0
no lime	36.7	3.55	10.3	NA	5.9
PKS(-N)					
Lime	37.1	3.64	10.2	NA	7.1
no lime	36.3	3.56	10.2	158	5.8

Table 4 Continued:

Soil/Rotation	Total C (Mg ha^{-1})	Total N (Mg ha^{-1})	C:N ratio	Total S (kg/ha)	pH
WF					
Control					
Wheat	22.7	2.16	10.3	NA	7.3
Fallow	27.1	2.64	10.2	85	7.3
Manure					
Wheat	41.7	5.20	8.0	317	6.9
Fallow	38.9	4.58	8.5	NA	6.8
NPKS					
Wheat	26.3	3.09	8.5	NA	6.7
Fallow	27.7	3.04	9.6	NA	7.0
NPK(-S)					
Wheat	22.3	2.19	8.5	NA	6.3
Fallow	25.3	2.40	9.1	NA	7.1
PKS(-N)					
Wheat	19.3	1.90	10.2	NA	6.9
Fallow	21.3	2.08	10.3	85	7.0

3. Results and Discussion

3.1. Laboratory incubation experiment

A summary of the statistical analysis for each response variable is presented in Table 5. Soil (main plot) and fertilizer (subplot) treatments were significant for Total N_2O efflux, adsorbed S, P, K and Al. Only the fertilizer (subplot) treatment was significant for total adsorbed plant available N (nitrate + ammonium). There were no significant soil-by-fertilizer interactions. Plots showing the means ± standard error for selected soil-treatment combination for selected response variables are given in Figures 2 through 6.

Table 5: Summary of ANOVA (GLM) analysis on response variables. P values less than or equal to 0.05 are in bold-face and indicate a statistically significant treatment effect.

	Treatment Effect (P-Value)		
Response Variable	**Soil Management History (main plot)**	**Fertilizer Treatment (sub-plot)**	**Soil X Fertilizer Interaction**
Total N_2O efflux over 12 weeks (log transformed)	**<0.001**	**0.013**	0.749
Total CO_2 efflux over 12 weeks	0.109	0.625	0.146
Total adsorbed plant available N over 12 weeks	0.152	**<0.001**	0.402
Total adsorbed sulfate S over 12 weeks	**0.002**	**<0.001**	0.114
Total adsorbed available P over 12 weeks (log transformed)	**<0.001**	**0.001**	0.911
Total adsorbed available K over 12 weeks	**0.017**	**<0.001**	0.434
Total adsorbed Al over 12 weeks	**0.052**	**0.008**	0.783

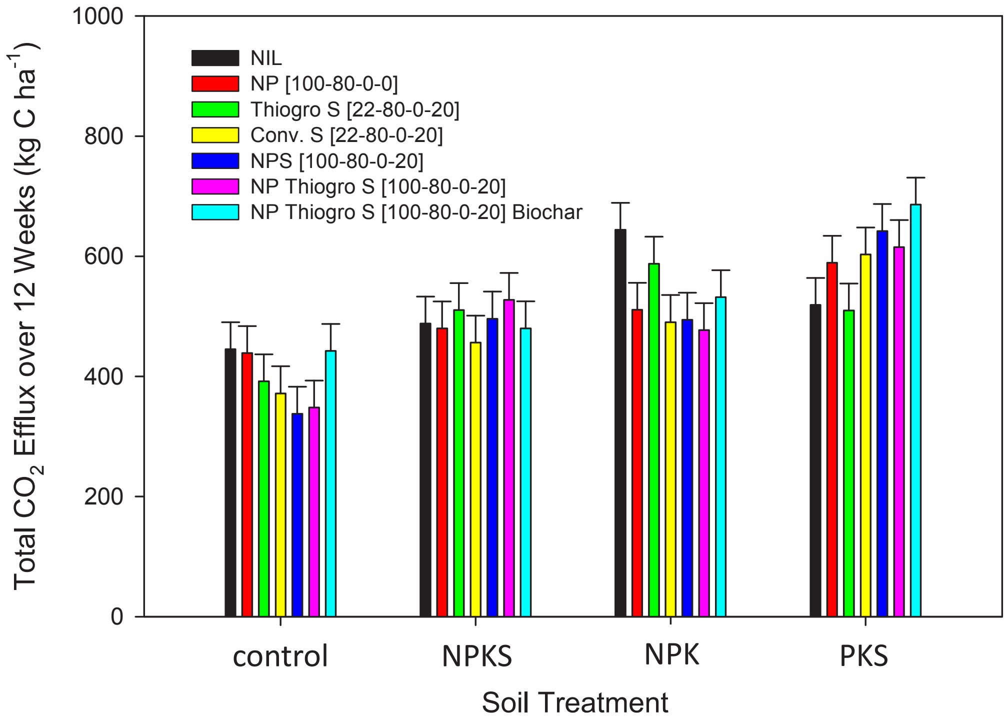

Figure 2: Summary of total CO_2 efflux (kg C ha^{-1}) as a function of soil management history and fertilizer treatment. The error bars represent the ANOVA-generated standard error. There were no significant soil or fertilizer effects.

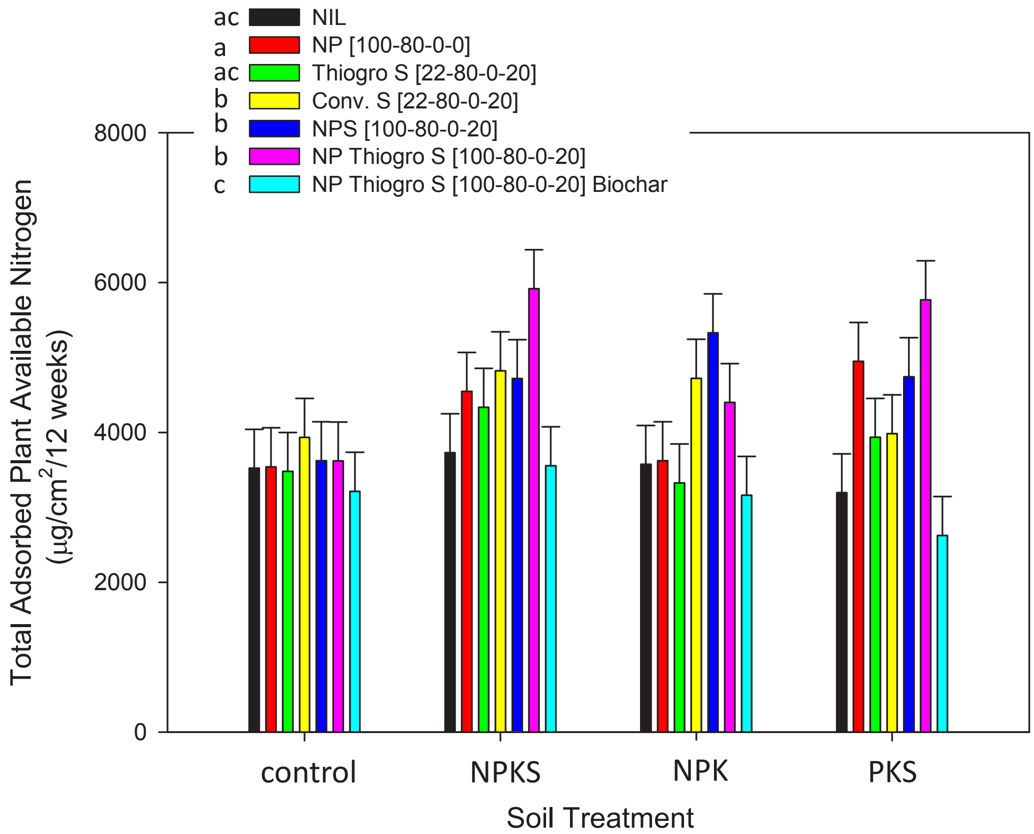

Figure 3: Total PRS-adsorbed plant available nitrogen (μg N/cm^2/12 weeks) as a function of soil management history and fertilizer treatment. The error bars represent the ANOVA-generated standard error. Letters beside the fertilizer subplots (legend) indicate least-significant difference comparison of means. Subplots with the same letter(s) indicate that their means are not significantly different from each other. Soil main plot and soil management-by-fertilizer interactions were not significant (Table 2) so comparison of means is within subplots (averaged over all main plots).

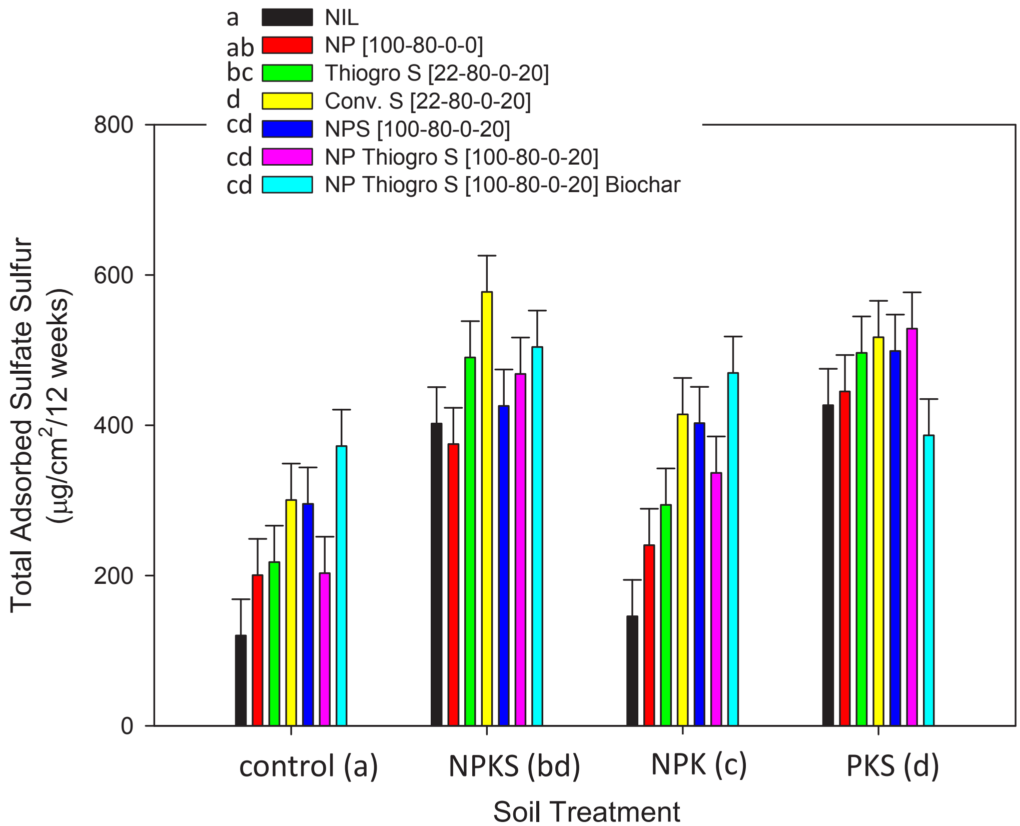

Figure 4: Total PRS-adsorbed plant available sulphur (sulfate, µg S/cm^2/12 weeks) as a function of soil management history and fertilizer treatment. The error bars represent the ANOVA-generated standard error. Letters beside the soil management main plots (x-axis) and fertilizer subplots (legend) indicate least-significant difference comparison of means. Main plots or subplots with the same letter(s) indicate that their means are not significantly different from each other. Soil management-by-fertilizer interactions were not significant (Table 2) so comparison of means is within main plots (averaged over all subplots) or within subplots (averaged over all main plots).

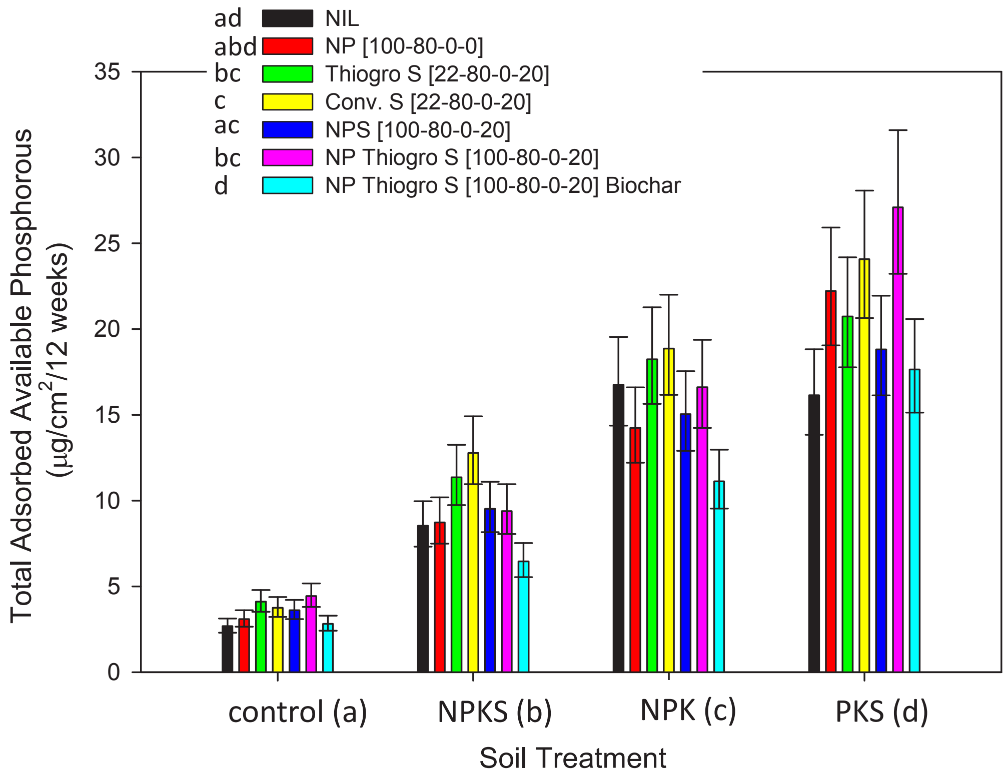

Figure 5: Total PRS-adsorbed plant available phosphorous (µg P/cm^2/12 weeks) as a function of soil management history and fertilizer treatment. The error bars represent the ANOVA-generated standard error. Letters beside the soil management main plots (x-axis) and fertilizer subplots (legend) indicate least-significant difference comparison of means. Main plots or subplots with the same letter(s) indicate that their means are not significantly different from each other. Soil management-by-fertilizer interactions were not significant (Table 2) so comparison of means is within main plots (averaged over all subplots) or within subplots (averaged over all main plots).

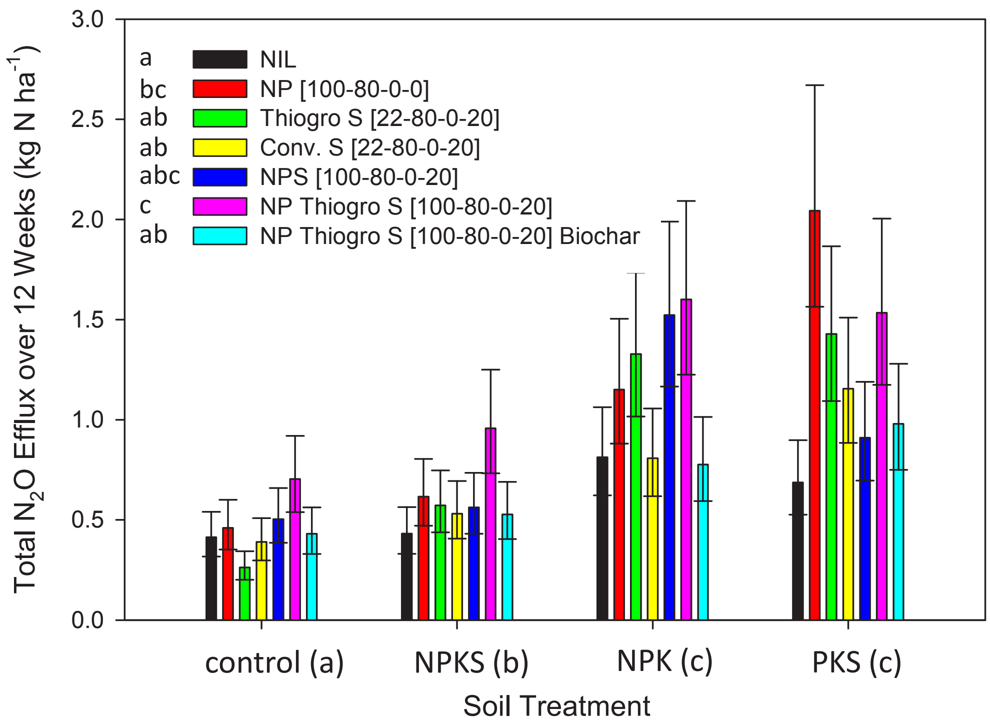

Figure 6: Total N_2O efflux (kg N ha^{-1}) as a function of soil management history and fertilizer treatment. The error bars represent the ANOVA-generated standard error. Letters beside the soil management main plots (x-axis) and fertilizer subplots (legend) indicate least-significant difference comparison of means. Main plots or subplots with the same letter(s) indicate that their means are not significantly different from each other. Soil management-by-fertilizer interactions were not significant (Table 2) so comparison of means is within main plots (averaged over all subplots) or within subplots (averaged over all main plots).

Both fertilizer application history (soil main plot) and fertilizer treatments (subplot) affected nitrous oxide fluxes and nutrient supply (Table 5; Figs. 2-6). Increases in the amount of PRS-adsorbed nutrients/ions represent an increase in the amount of that ion that was apparently available in the soil for plant uptake. The availability is only apparent because the PRS probes are not plant roots – they differ in geometry and they are inorganic – but they act as an ion sink like plant roots. Further, in this incubation study, there were no plants grown in the jars, so the PRS-adsorbed ions should be considered apparent; that is, they are only potentially available to plants

As indicated in Table 5, soil management history and fertilizer addition did not significantly influence the total CO_2-C efflux over the 12 weeks. However, the P-value for the mainplot (soil management history) effect is still quite low (0.11) and there are some noticeable trends between the

the control soil. Of the soils with long-term fertilizer additions, the soil with balanced fertilization (NPKS) had slightly lower CO2-C emissions than the NPK or PKS soils even though the NPKS soil has a greater amount of total organic carbon (Table 2). One possible explanation is that the greater amount of TOC in the NPKS soil is more stable (i.e., protected by soil aggregates).

Most N that is stored in the soil is stored as organic compounds in the soil organic matter or in the soil microbial biomass. This organic N can be mineralized (oxidized) to NH_4^+ which is subsequently nitrified to NO_3^{2-}. Soil management history did not significantly influence the amount PRS-adsorbed plant available N (NO_3^{2-} + NH_4^+; Table 5; Fig. 3). Surprisingly, in the control soil, additional fertilizer N did not increase the amount of N adsorbed onto the PRS probes, suggesting that that added N was immobilized, by the microbial biomass, volatilized as NH_3 or lost as nitrous oxide. In the NPKS, NPK and PKS soils, added fertilizer increased the amount of PRS-adsorbed N significantly.

Both soil management and fertilizer treatments had significant impacts on PRS-adsorbed soil available S (sulfate; Fig. 4)). Soils having had long-term applications of S fertilizer, showed higher levels of available S in the soil. Additions of sulfate and elemental S fertilizers resulted in increases in plant available S. It is not clear at this point, how much of the added elemental S was oxidized to sulfate and then adsorbed to the PRS probes.

Available phosphorous was strongly influenced by soil management history and by fertilizer treatment (Table 5 and Fig. 5). The phosphate ion is the available form of phosphorous. Soils with long-term application of P fertilizers showed significantly more available P than the control soil (Fig. 4). Each of the NPKS, PKS and NPK treatments have received 22 kg P ha^{-1} since 1980, and 6, 9 and 6 kg P ha^{-1} from 1930 - 1980. Therefore the differences between these soils are not a result of different long-term P fertilizer application rates. These results suggest the importance of balanced fertilization on P uptake. The source of the PRS-adsorbed P is likely residual P in the soil and P from added fertilizers. The balanced NPKS soil has low residual P because crop yields and crop uptake of all added nutrients has been greatest because of balanced fertilization. In the PKS and NPK soils, macronutrient deficiencies have not been offset by added nutrients. Therefore, the PKS and NPK soils show greater levels of PRS-adsorbed P because long-term P uptake by crops is lower in these soils because of unbalanced fertilization.

Soil fertilization history and fertilizer treatment significantly influenced to N_2O efflux (kg N ha^{-1}; Table 5; Fig. 6). It is interesting to note that the soil without long-term fertilizer N additions (PKS) or long-term fertilizer S additions (NPK) had greater total N_2O emissions that the soil with long-term balanced fertilizer additions (NPKS). As noted in the introduction, there is generally a positive relationship between the rate of nitrogen fertilization and nitrous oxide emissions. In this case, soil management history appears to significantly influence the relationship between N fertilizer application and nitrous oxide emissions.

In the case of the control soil with no long-term fertilizer nutrient additions, addition of N fertilizers did not increase the amount of PRS-adsorbed plant available N or nitrous oxide emissions. This is likely because applied N was immobilized or volatilized. Another possible explanation is that the control soil is very low in urease because it hasn't had any long-term urea additions and so added urea wasn't converted to and plant available forms of N.

For the soils with long-term fertilizer additions, there are significant differences in the nitrous oxide production as a result of added N fertilizer. The PKS, NPK and NPKS soils both showed increased N_2O emissions over the control soil as a result of added N fertilizer as expected. Because the incubation was conducted under aerated conditions, the nitrous oxide production process is hypothesized to be nitrifier denitrification (i.e., the use of nitrite as an electron acceptor during nitrification) and then converted to N_2O. The mean total N_2O efflux increases in the order of NPKS, NPK and PKS which is the decreasing order for total organic carbon levels in the soil, suggesting that immobilization of applied nitrogen may partly explain the decrease N_2O efflux in soils with higher total organic carbon, but the C:N ratios of these soils don't differ very much (table 4).

Another possible explanation for the low N2O emissions from the NPKS soil is interaction between nitrogen and sulphur cycling. Sulfur oxidizing bacteria may use nitrite as an electron acceptor to produce N_2 gas (Hasegawa et al. 2000) and this process has been shown to reduce dissolved N_2O levels in the pore waters of estuarine sediments. It is not clear why this process may have been active in the NPKS soil, but not the PKS soil, but there is some evidence to suggest it was occurring in selected fertilizer treatments of the PKS soil (i.e., NP-Thiogro S treatment emissions are lower than NP treatment emissions). There may have been some variations in packing of the soil in the incubation microcosms creating variability in anaerobic microsites. The NPKS soil has had long-term applications of both elemental S and urea N and therefore likely has the microbial community required for such coupled complex cycling of N and S.

3.2. Field Gas Flux Measurements

A summary of the 2013 growing season gas flux measurements is presented in Fig. 7. Despite lack of replication, there are some apparent trends worth noting. In terms of rotation, cumulative growing season N_2O fluxes were highest in the un-limed 5-year rotation, followed by the limed 5-year rotation, WF-wheat phase and WF-fallow phase.

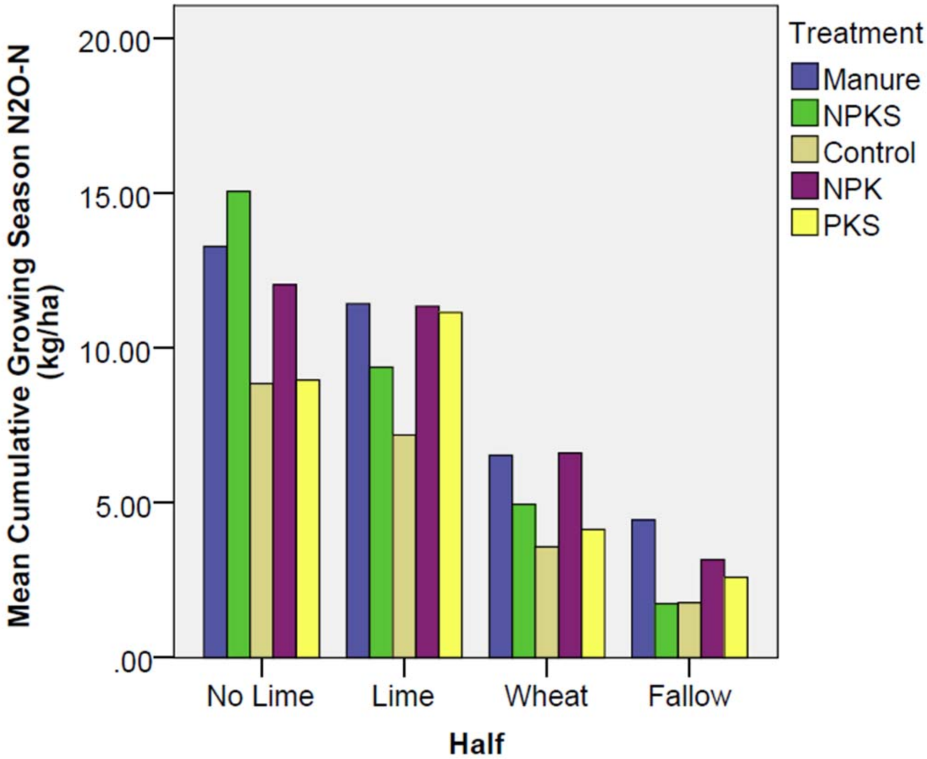

Figure 7: Summary of cumulative N2O emissions in the 2013 growing season on selected treatments of the Breton Classical Plots.

Despite lower N fertilizer application rates on wheat in the WOBHH rotation compared to the WF rotation, there is likely significant levels of soil N from the previous 2 years of alfalfa-brome hay. The N fixed in the roots and remaining above ground residue of the alfalfa is likely being mineralized and contributes to greater N_2O fluxes compare to the wheat-fallow rotation. The cumulative N_2O losses from the WOBHH rotation are significant especially in the un-limed halves of the plots, up to 15 kg N/ha which is 30% of applied fertilizer in the wheat phase.

For plots with lime additions (lime, wheat and fallow in Fig. 7), consistently showed the same ranking of cumulative nitrous oxide emissions; namely, Manure > NPK > PKS > NPKS > Control. In the laboratory incubation, the relative ranking was NPK=PKS > NPKS > Control. The soils for the laboratory experiment were taken from series C (Fig. 1) and the field measurements in 2013 were taken from series D (Fig. 1). Therefore, it appears that the fertilizer history has a consistent influence on N_2O emissions over space. Further, the agreement in the results of the lab and field experiments lends further credence to the possibility that the hypothesized interactions between N and S cycling in soils may reduce N_2O emissions in when elemental S fertilizer is applied along with N fertilizer and the soil has a long-term history of balanced NPKS fertilization.

Fig. 8 summarizes the temporal dynamics of the 2013 growing season precipitation, air temperature, reference soil moisture (from weather station compound) and N_2O fluxes for the limed and un-limed halves of the WOBHH-Wheat phase plots. There is a spike in N_2O fluxes right after fertilizer application (broadcast and incorporate) and seeding, especially in the un-limed half. Subsequent increases in N_2O fluxes are associated with soil drying following significant precipitation events. This is consistent with reports studies cited by Braker and Conrad (2001). Under very wet soil conditions, ammonium oxidation is inhibited resulting in low levels of nitrite, the precursor to N_2O when produced by nitrifiers. Once the soil has dried, ammonium oxidation proceeds, nitrite is produced and N_2O production increases.

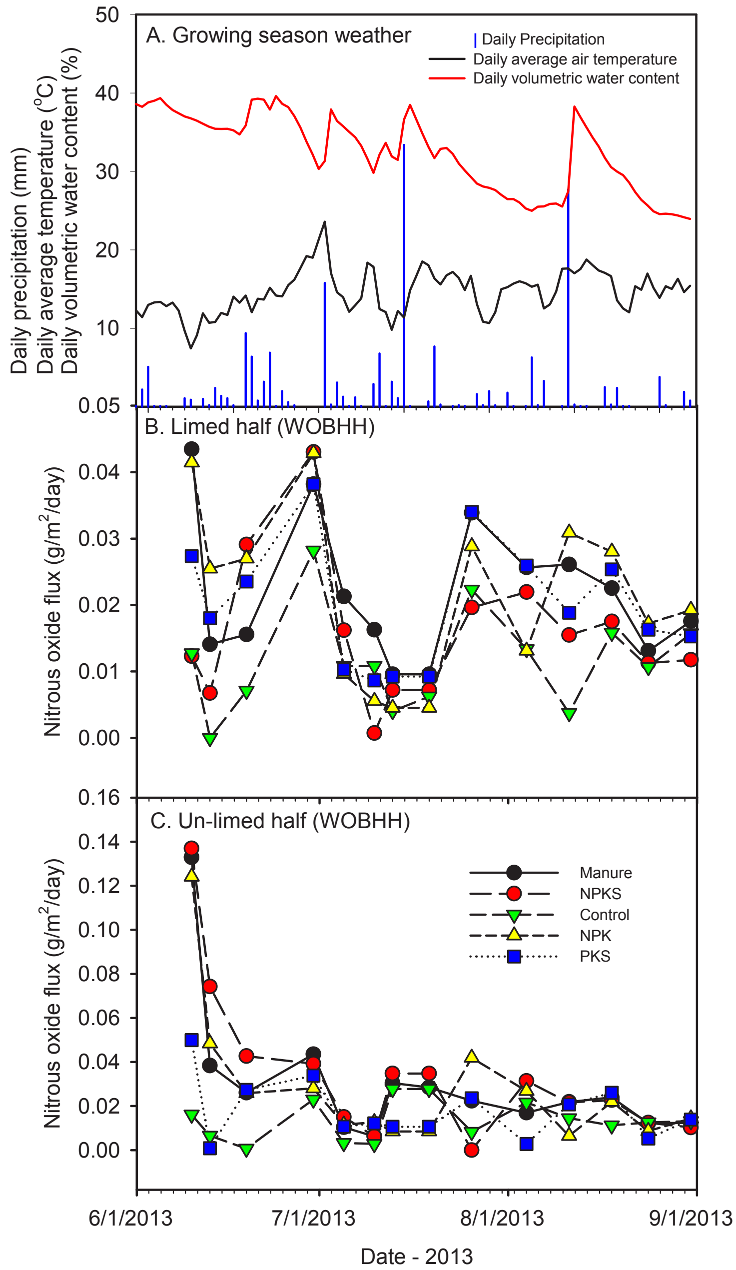

Figure 8: Precipitation, air temperature, soil moisture (at 5 cm depth) and N2O fluxes on selected treatments of the WOBHH rotation of the Breton Classical Plots in the 2013 growing season.

The N2O flux spike following fertilization is much higher in the un-limed plots and cumulative growing season N2O emissions are significantly correlated to soil pH (Fig. 9). This is consistent with the hypothesis put forward by Wrage et al. (2001); namely, under low pH conditions nitrifier denitrification is a more thermodynamically stable process. Therefore, management of soil pH likely plays a significant role in reducing nitrous oxide emission from well-aerated soils.

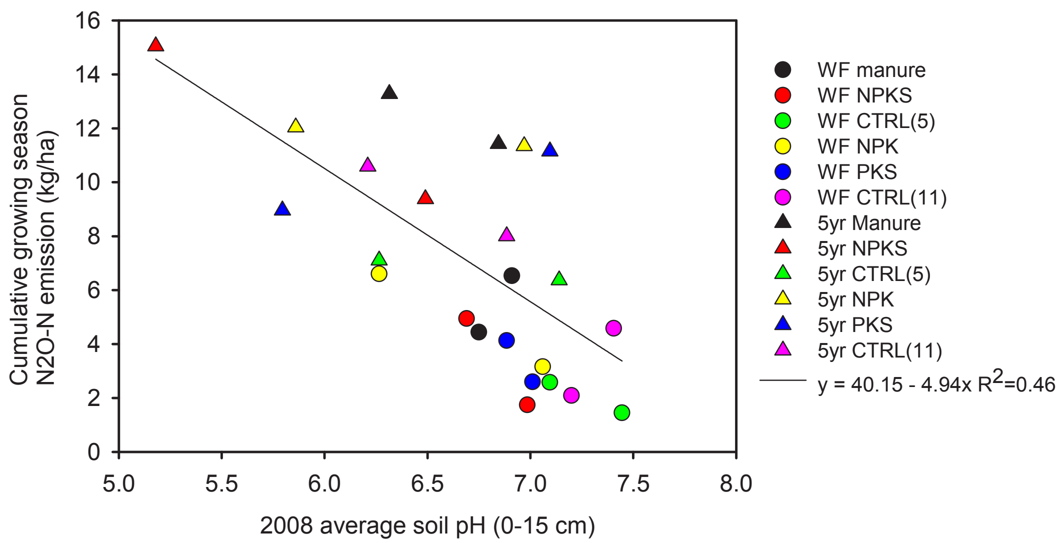

Figure 9: Relationship between soil pH and 2013 cumulative N2O emissions on the Breton Classical Plots.

4. Conclusions and Generated Hypothesis

The main objective of this research was to assess the greenhouse gas emission response of soils with different fertilizer application histories to contemporary fertilizer applications. Sulfur-deficient Soils from the University of Alberta Breton Plots were used for this research because they have been under consistent management for 30 years. For both lab and field measurements, soil with a history of balanced fertilization(NPKS) had consistently lower (5 to 20% lower) cumulative nitrous oxide emissions compared to soils with long-term NPK or PKS fertilization (i.e., one major macronutrient missing) when soil pH was greater than 6.0. This effect is hypothesized to be a result of an interaction between S and N cycling in the soil whereby nitrite is used as an electron acceptor by S-oxidizing bacteria during elemental S oxidation. In the field, lower N_2O fluxes on treatments with balanced fertilization may also be a result of greater plant N uptake.

The results from this research are interpreted assuming that the major process contributing to N_2O production and emissions from these soils is nitrifier denitrification whereby autotrophic nitrifying bacteria use nitrite - produced from ammonium oxidation - as an electron acceptor during their metabolism which results in N_2O production. This process has been shown to be a major contributor to N_2O production in agricultural soils, especially soils with high N, low organic carbon, moderate aeration and low pH (Braker and Conrad, 2011) and the soils at the Breton Plots generally fit this description.

Future research will focus on the design and execution of field and laboratory experiments to test the S and N cycling interaction hypothesis. A summary of the key findings of this research with respect to the 4R/NERP frameworks is presented below.

4R/NERP Principle	Application of results from this research
Right Product	4) In soils where nitrifier denitrification is the main N_2O production process, the banding of elemental S with ammonium-based fertilizers may help to reduce nitrous oxide emissions because S-oxidizing bacteria can use nitrite as an electron acceptor and the end result is N_2 instead of N_2O. This process was not directly observed in this research, but has been observed in other systems and is a hypothesis generated by the observations that soils with long-term NPKS fertilization had 5 – 20% lower N_2O-N emissions than soils with long-term NPK or PKS fertilization. This result also demonstrates the importance of long-term balanced fertilization on residual soil nutrient levels. If this hypothesized N-S interaction is active, soil fertilizer N is still lost to the environment in a more benign form (i.e., N fertilization use efficiency is not likely to be increased). 5) This research observed that soils with low pH (acidic) had significantly greater N_2O emissions than soils with higher pH. At the Breton plots,

	cumulative growing season emissions increased by 5 kg N2O-N/ha for every 1.0 decrease in pH. Liming is recommended to maintain soil pH above 6.0. 6) Ammonia-based fertilizers are recommended because they are less susceptible to leaching. However, this research showed that in soils where the dominant N_2O production process is nitrifier denitrification, ammonia-based fertilizers may not be the right product because oxidation of ammonium produces nitrite which is the precursor of nitrous oxide production in this process. The use of nitrification inhibitors might help to significantly N_2O emissions where nitrifier denitrification is the main N_2O production process.
Right Rate	This research showed that high levels of residual N from previous years can significantly increase nitrous oxide fluxes at Breton. This was shown by the observation that N_2O fluxes in wheat following two years of alfalfa-brome in the WOBHH rotation were up to 3X greater than the wheat-fallow rotation despite a much lower N fertilizer application rate (50 kg N/ha for WOBHH; 90 kg N/ha for WF). Even though N fertilizer is not applied in the hay phase of this rotation, alfalfa contributes to increased N levels in the soil through N-fixation and through N-rich residues which are ploughed under prior to seeding annual crops. Coupled with applications of N fertilizer to subsequent annual crops, it would appear that this rotation has resulted in the accumulation of excess residual N over the long-term. In order to avoid excess residual N, fertilizer N recommendations appropriate for the crops in the rotation based soil tests are recommended.
Right Place/Right time	This research observed that significant losses of N fertilizer to N_2O can occur following application if fertilizer is broadcast and incorporated especially in acidic soils. The method for fertilizer applications at the Breton Plots is to broadcast and incorporate with tillage prior to spring seeding. This application of N fertilizer to moist soil followed by tillage, increases soil oxygen levels, soil temperature and microbial activity. As a result, the urea N fertilizer is quickly transformed to ammonium which is then oxidized to nitrite which is susceptible to nitrifier denitrification. Nitrous oxide losses were greater in soils with low pH. Banding N fertilizer in the spring or late fall slows down the transformation of N fertilizers because the soil is not disturbed to the same extent during banding compared to incorporation with tillage. Banding fertilizers decreased N_2O emissions.

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