4R NUTRIENT STEWARDSHIP:

Nitrous oxide emissions from corn as affected by timing and rate of nitrogen fertilizer application

CANADIAN FERTILIZER INSTITUTE
INSTITUT CANADIEN DES ENGRAIS

4R nutrient stewardship

Final Report to Canadian Fertilizer Institute — August 2010-2013

Project Title:

Nitrous oxide emissions from corn as affected by timing and rate of nitrogen fertilizer application

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Duration of Project

September 1, 2010, to August 31, 2013

Brief Project Description

Nitrous oxide (N_2O) emissions are estimated to comprise the most important source of greenhouse gas (GHG) emissions associated with growing corn. Applying nitrogen fertilizer at a rate and time to match corn needs are considered management practices that can lead to significant reductions of on-farm GHG emissions. However, additional research is needed to quantify the magnitude of N2O emission reduction associated with these practices. The research conducted as part of this project addressed this knowledge gap by providing measurements of N2O emissions from corn in a comprehensive field trial designed to evaluate the long-term effects of timing and rate of fertilizer N on grain corn yield potential. This project complemented on-going research on the environmental footprint of corn and life-cycle analysis of corn residue as feedstock for bioenergy. Research Activities Soil gas chambers were used to measure nitrous oxide emissions in 2011 and 2012 from a 10-year field trial, whose project leader was Bill Deen/ with project leader Bill Deen, initiated at the Elora Research Station in Elora, Ontario, in 2009 and funded by IPNI. A total of 12 treatments (3 rates x 2 timings x 2 histories) were studied with one soil gas chamber deployed on each of the four replicated treatments (Table 1 and 2). The two N fertilizer histories consist of applying the N application rates and timing combinations on plots that receive 145 kg N ha^{-1} in the previous year (short-term), and, on a different set of plots, repeating each of the same N application rates and timing combinations on each plot over the duration of the trial (long-term). N2O fluxes were measured approximately once per week for a total of 28 times from May 13, 2011, to October 17, 2011, and 24 times from May 3, 2012, to October 4, 2012. A view of the sampling procedure is shown in Figure 1. Supporting measurements consisted of soil water content, soil temperate, and soil nitrate and ammonium concentrations. Detailed explanation of methods are given in Roy et al. (2014) (attached).

Table 1: Timing treatments studied during this research with 4R practice indicated.

N at planting	UAN injected into inter-rows immediately following planting May 12, 2011/May 2, 2012
N at the 8th leaf stage (4R)	UAN injected into inter-rows ~1 month after planting June 15, 2011/June 13, 2012

Table 2: Rate treatments studied during this research with 4 R practice indicated. ONC = Ontario Nitrogen Calculator Rate, 145 kg N ha^{-1}

	Starter (kg N ha^{-1})	At planting/Side-dress (kg N ha^{-1})	Total (kg N ha^{-1})
ONC – 20%	30	0	30
ONC (4R)	30	115	145
ONC – 150%	30	188	218

Fig. 1: View of experimental site and sampling of soil gas chambers.

Summary of Results

Highest N_2O emissions induced by nitrogen fertilizer application typically occur in April-May when soil moisture is relatively high and corn N uptake low. This effect was observed in both study years. Difference between study years were also observed with the growing season of 2011 being significantly wetter than 2012, and soil water conditions significantly higher after planting compared to the 8th leaf stage in 2012 (Fig. 2). As a result, emissions were larger in 2011 compared to 2013 (Fig. 3). Delaying N application to V8 stage (4R), and applying the recommended rate (145 kg N ha^{-1}; 4R) significantly reduced N_2O emissions in 2011, but no significant effect was observed in

2012 (Fig. 3). Overall delaying the bulk of N fertilizer application until the V8 stage (4R) reduced N_2O emissions by 58% in a typical wet spring. The highest N rate (218 kg N ha^{-1}) increased grain yield only by 6% but N2O emissions by 64% revealing the importance of using appropriate N rate (4R) (Fig. 4). Corn grain yield was not significantly different between the side-dress timing of fertilizer application in both years (Fig. 4).

Matching amount and timing of application to crop uptake, an integral part of the 4R Nutrient Stewardship program, has the potential to significantly reduce N2O emissions under typical Ontario early growing season conditions.

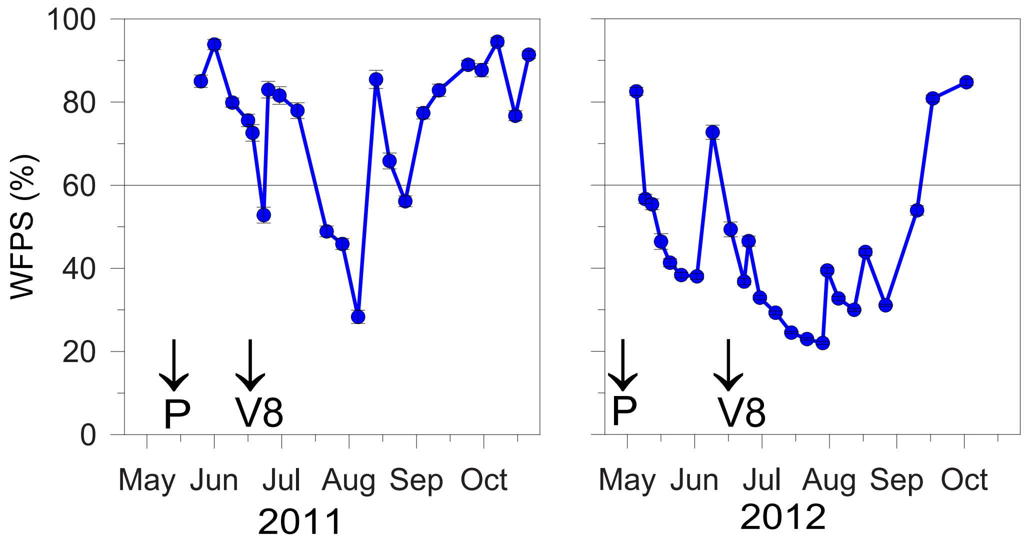

Fig. 2: Water filled pore space (WFPS) during the corn growing season in both study years. Values of WFPS larger than 60% are conducive for soil N_2O production. Arrows show timing of fertilizer application at planting (P) and 8^{th} leaf stage (V8).

Conclusions:

In a typical Ontario wet spring, N applied as side-dress at the 8_{th} leaf stage in corn compared to at planting resulted in a significant reduction in N_2O emissions at the recommended (145 kg N ha^{-1}) and above recommended (218 kg N ha^{-1}) N application rate. Corn grain yield was not affected by timing and as a result, N_2O emission intensity was also significantly reduced when N fertilizer application was timed to better coincide with plant N uptake. Water-filled pore space was the main controlling factor on N_2O growing season emissions, hence a large reduction in emissions can be expected in years when WFPS is lower following side-dress compared to following planting. Lower WFPS in June is usually present in Ontario due to higher evapotranspiration as induced by higher temperature and plant water uptake, and hence we suggest this management practice has potential for long-term N_2O emission reduction.

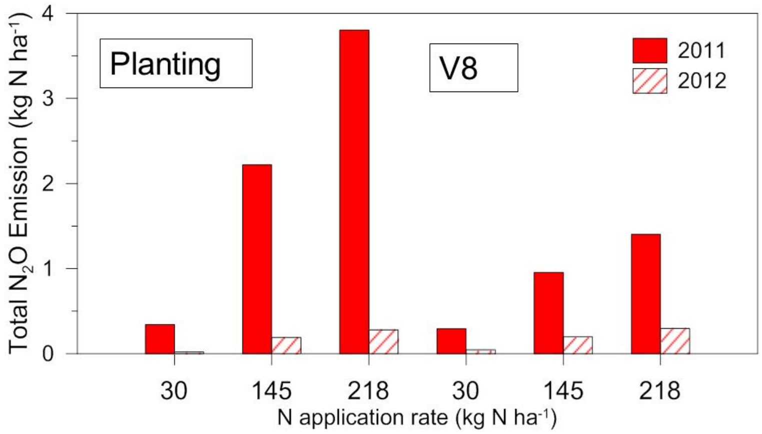

Fig. 3: Total nitrous oxide emissions over two growing seasons according to timing and rate of nitrogen fertilizer application. Values were averaged for the two history treatments as no significant effect was of observed.

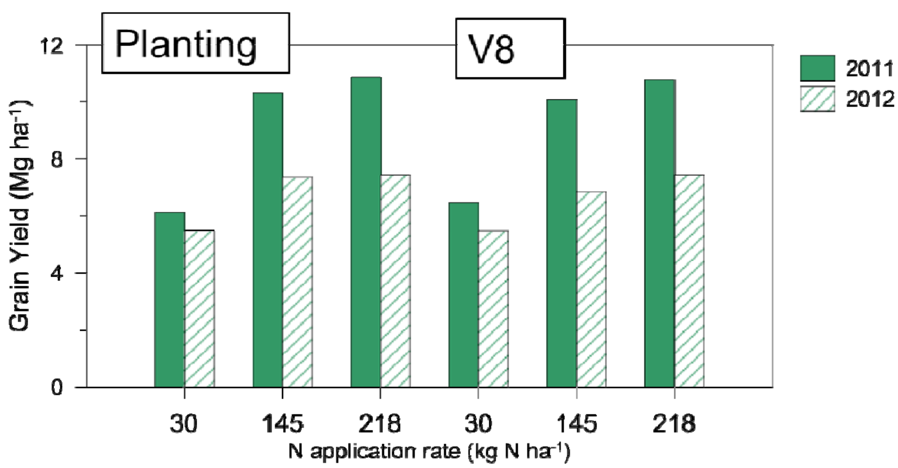

Fig. 4: Corn grain yield over two growing seasons according to timing and rate of nitrogen fertilizer application. Values were averaged for the two history treatments as no significant effect was of observed.

Leveraged Funds:

Graduate student Amal Roy is worked full-time on this project. He was the recipient of a $21,000 per year stipend for 3 years from the OMAFRA/University of Guelph agreement as of May 1, 2010. In addition, as advisor I am required to top up this amount at a rate of $5,000 per year. These funds came out of my OMAFRA/U of G project titled ‘Life Cycle Analysis Framework and Model for Assessing Life Cycle Impacts and Environmental Sustainability of Ontario Agricultural Feedstocks for Bioenergy’.

Nitrogen application rate, timing and history effects on nitrous oxide emissions from corn (*Zea mays* L.)

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[1]School of Environmental Sciences; [2]Department of Plant Agriculture, University of Guelph, 50 Stone Road East, Guelph, Ontario, Canada N1G 2W1; and [3]International Plant Nutrition Institute, 18 Maplewood Drive, Guelph, Ontario, Canada N1G 1L8.

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Roy, A. K., Wagner-Riddle, C., Deen, B., Lauzon, J. and Bruulsema, T. 2014. **Nitrogen application rate, timing and history effects on nitrous oxide emissions from corn (*Zea mays* L.)**. Can. J. Soil Sci. **94**: 563–573. Nitrous oxide (N_2O) emissions resulting from application of nitrogen (N) fertilizer contribute to the greenhouse gas footprint of corn production. In eastern Canada, corn is a major crop with most N fertilizer applied pre- or at planting. This timing of application results in a lack of synchrony of soil N supply and crop N demand. Matching the amount and timing of application to crop uptake has been suggested as a mitigation measure to reduce N losses, and is an integral part of the 4R Nutrient Stewardship program. This study examined the effect of timing, rate and history of urea-ammonium nitrate application on N_2O emissions in corn in 2011 and 2012 at Elora, ON, Canada. Treatments were three N rates (30, 145 and 218 kg N ha^{-1}); two timings (N injected in mid-row at planting and at the 8th leaf stage, V8); two histories (short-term: applying N rate treatments on plots that had received 145 kg N ha^{-1} in the previous year, and long-term: applying the same N rate to a given plot over the duration of the trial). N_2O emissions were measured using static chambers. History of N application did not have an effect on N_2O emissions or grain yield. In both years, cumulative N_2O emissions during the growing season and corn yields increased significantly with increasing N application rates. In 2011, cumulative N_2O emissions were significantly lower when N was applied as side-dress at V8 (0.88 kg N ha^{-1}) compared with planting (2.12 kg N ha^{-1}), with no significant impact on corn grain yield (average 9.1 Mg ha^{-1}). In contrast, in 2012, limited rainfall reduced both N_2O emissions and corn grain yield, and neither N_2O emission (average 0.17 kg N ha^{-1}) nor grain yield (average 6.7 Mg ha^{-1}) was affected by timing of N application. Applying N as side-dress at V8 instead of at planting and using the recommended N rate were shown to be effective N_2O emission mitigation practices without affecting corn yield during a typical wet spring in Ontario.

Key words: Nitrous oxide emissions, corn yield, application timing, N fertilizer history, side-dress application

Roy, A. K., Wagner-Riddle, C., Deen, B., Lauzon, J. et Bruulsema, T. 2014. **Conséquences du taux, du moment et des antécédents d'application des engrais azotés sur les émissions d'oxyde nitreux par le maïs (*Zea mays* L.)**. Can. J. Soil Sci. **94**: 563–573. Les dégagements d'oxyde nitreux (N_2O) attribuables à l'application d'engrais azotés (N) ajoutent à l'empreinte du maïs sur les gaz à effet de serre. Le maïs est une importante culture dans l'est du Canada et la majeure partie des engrais N est appliquée aux semis ou avant, si bien qu'il y a discordance entre la quantité d'azote présente dans le sol et celle dont la plante a besoin pour croître. En vue d'atténuer les pertes d'azote, d'aucuns suggèrent qu'on ajuste la quantité d'engrais et le moment de leur application avec le taux d'absorption de la culture, mesure qui fait partie intégrante du programme *4R Nutrient Stewardship*. Dans le cadre de cette étude, les auteurs ont examiné les conséquences du moment, du taux et des antécédents d'application de l'urée-nitrate d'ammonium sur la quantité de N_2O libérée par le maïs en 2011 et en 2012, à Elora, en Ontario (Canada). Les traitements étaient les suivants : trois taux d'application d'engrais N (30, 145 et 218 kg de N par hectare); deux moments d'application (injection au milieu du rang aux semis et au stade de la huitième feuille, V8); deux antécédents d'application (à court terme : taux d'application précités sur des parcelles bonifiées avec 145 kg de N par hectare l'année antérieure; à long terme : application du même taux d'engrais N à une parcelle pendant la durée complète de l'expérience). Les émissions de N_2O ont été mesurées grâce à des chambres statiques. Les antécédents d'application n'ont aucun effet sur les dégagements de N_2O ni sur le rendement grainier. Les deux années de l'étude, les émissions cumulatives de N_2O relevées pendant la période végétative et le rendement du maïs ont augmenté de manière significative avec le relèvement du taux d'application des engrais N. En 2011, les émissions cumulatives de N_2O se sont révélées significativement plus faibles quand l'engrais N a été épandu en bandes latérales au V8 (0,88 kg de N par hectare) plutôt qu'aux semis (2,12 kg de N par hectare), sans impact majeur sur le rendement grainier (9,1 Mg par hectare, en moyenne). En 2012, en revanche, des précipitations restreintes ont diminué à la fois les émissions de N_2O et le rendement grainier, sans que le moment où l'engrais a été appliqué exerce une influence quelconque sur les dégagements du gaz (0,17 kg de N par hectare, en moyenne) ou le rendement grainier (6,7 Mg par hectare, en moyenne). Épandre l'engrais en bandes latérales au V8 plutôt qu'aux semis, en respectant le taux recommandé, semble constituer une mesure efficace pour atténuer les émissions de N_2O sans que le rendement du maïs en pâtisse lors d'un printemps humide, typique à ceux de l'Ontario.

Mots clés: Émissions d'oxyde nitreux, rendement du maïs, moment d'application, antécédents de fertilisation N, épandage en bandes latérales

Human-induced emissions of the potent greenhouse gas nitrous oxide (N_2O) are primarily related to agricultural soils [Intergovernmental Panel on Climate Change (IPCC) 2007]. The production of N_2O in soil is a function of nitrification and denitrification mediated by soil microbes (Robertson and Groffman 2007; Freing 2012). Factors that regulate denitrification and nitrification, and hence N_2O production in soil, include available carbon, inorganic N, and oxygen, all of which are affected by soil water, porosity, and aggregate structure (Robertson and Groffman 2007).

The application of inorganic N fertilizers to agricultural soils is considered to be the main source of N_2O emission from soils (Eichner 1990; Matthews 1994; Bouwman et al. 2002; Rochette et al. 2008b). In eastern Canada, corn is a major crop (Statistics Canada 2013), and a large proportion of its greenhouse gas emissions (47%) is due to N fertilizer input (34% from soil N_2O; 13% from fertilizer production and supply) (Jayasundara et al. 2014), with most N fertilizer applied pre- or at planting. This timing of application results in a lack of synchrony of soil N supply and crop N demand (Cassman et al. 2002; Fageria and Baligar 2005), and relatively low nitrogen use efficiency for farmer-managed fields, rarely exceeding 50% (Raun and Johnson 1999; Roberts 2008). Miller et al. (2012) reported that the efficiency of N use by corn may be improved by delaying N application from planting to early vegetative stages just prior to the rapid growth phase (approximately the 6th to 8th leaf stage in corn, V6 to V8).

Timing of N application may also affect N_2O emission. Robertson and Vitousek (2009) suggested that further study on reduction of N_2O emission in agricultural systems should focus on improving nitrogen use efficiency by applying fertilizer at a time that coincides with crop demand. Matching amount and timing of application to crop uptake has been suggested as a mitigation measure to reduce N_2O emissions, and is an integral part of the 4R Nutrient Stewardship program (Bruulsema et al. 2009). Many fertilizer N timing studies have compared the effect of fall versus spring application of N fertilizer on N_2O emissions (Hao et al. 2001; Hultgreen and Leduc 2003; Grant et al. 2004; Phillips et al. 2009; Millar et al. 2010), but few studies have compared planting versus vegetative growth stage applications. Zebarth et al. (2008) found NH_4NO_3 applied to barley at planting in a band compared with surface broadcasting at the 6th leaf stage resulted in similar cumulative N_2O emissions. However, timing was confounded with method of application in their study. Drury et al. (2012) reported 33% lower N_2O emissions associated with urea applied as side-dress compared with at planting under conventional tillage in corn. In contrast, Ma et al. (2010) reported higher N_2O emissions associated with side-dress application of N fertilizer versus pre-plant in corn, but cautioned that the short monitoring period (28 d) may have missed important emission events and skewed results. Hence, given the importance of nitrogen use in corn production, additional studies on the effect of timing of N application on whole growing season N_2O emissions are needed.

Applying N in excess of crop requirement increases soil NH_4^+-N and NO_3^--N concentrations in soils (Andraski et al. 2000). As a consequence, relatively higher N_2O emissions can occur when compared with applications at the required rate (Gregorich et al. 2005; McSwiney and Robertson 2005; Wagner-Riddle et al. 2007; Ma et al. 2010). In addition, continuous N application at high rates can lead to residual soil mineral N levels, which could affect N_2O emissions, but long-term effects of applying the same high N rates on corn and its interaction with timing have not been studied.

Gregorich et al. (2005) summarized data on N_2O emission as influenced by N application rates from eastern Canada and estimated a fertilizer-induced emission (FIE) factor (the proportion of N input converted to N_2O-N) of 1.19%. Rochette et al. (2008a) derived a regional FIE of 1.7% for the humid provinces of Quebec and Ontario, and developed a Tier II methodology for estimating emissions at the ecodistrict scale by scaling regional FIE using the ''precipitation to potential evapotranspiration'' ratio. These approaches scale N_2O emissions linearly as a function of N input, but studies have shown emission response to increasing N rate can be non-linear (Kim et al. 2013; McSwiney and Robertson 2005; Ma et al. 2010; Hoben et al. 2011; Van Groenigen et al. 2010). Studies evaluating how increasing N rates interact with other N management practices, such as timing and history of N application, are needed to fine-tune FIE factors.

The objectives of this study were: (1) to determine the effect of N application timing (planting vs. side-dress at V8) and N rate on N_2O emissions, and relate emissions to soil conditions, (2) to evaluate how short- and long-term history of N application rates to corn affect soil residual mineral N levels and N_2O emissions, and (3) to determine the effect of delaying N application to V8 and of N application history (and interaction with N rate) on FIE, corn yield and N_2O intensity (emission per kilogram of grain yield). Measurements were conducted during the growing season over 2 yr at a field site in eastern Canada in a factorial experiment that included timing, rate, and history of nitrogen application in corn.

MATERIALS AND METHODS

Experimental Site, Treatments and Design

This experiment was part of a trial under continuous corn production since 2009 at the University of Guelph, Elora Research Station (lat. 43°39′N, long. 80°25′W, 376 m elevation), Ontario, Canada. The soil at the experimental site is a Guelph loam (fine loamy, mixed, mesic Glossoboric Hapludalf, also described as orthic grey brown luvisol) (Hoffman et al. 1968) with 32% sand, 48% silt, 20% clay, and 4.5% soil organic matter, and at pH 7.7 (H_2O) (as analyzed by Agri-Food

Laboratories Inc.) and 1.30 ± 0.03 Mg m^{-3} soil bulk density. The site's 30-yr average annual precipitation is 874 mm and mean air temperature is 6.7°C.

The experiment was conducted in 2011 and 2012, respectively, the 3rd and 4th years of a long-term trial on fertilizer effects on corn yields initiated in 2009. Treatments included three N application rates (30, 145 and 218 kg N ha^{-1}, with 145 kg N ha^{-1} corresponding to the recommended rate), two application timings (planting and V8 stage) and two ''histories'' (short- and long-term), for a total of 12 treatments. The two N fertilizer histories consisted of applying N rates and timing combinations on plots that always received 145 kg N ha^{-1} in the previous year (short-term), and, on a different set of plots, repeating the same N application rates and timing combinations on each plot over the duration of the long-term trial. As an example of the history treatments, the short-term history plots for rate 30 kg N ha^{-1} received 145 kg N ha^{-1} in the previous year, while the long-term history plots for rate 30 kg N ha^{-1} had received 30 kg N ha^{-1} in each year since 2009. Hence the preceding 145 kg N ha^{-1} rate in the short-term history plots was meant to be an ''equalizer'' between rate treatments. The experimental design was a split plot with four replicates, where timing was the main plot within which history and rate were randomly assigned. The experimental unit consisted of a six-row plot with a row spacing of 0.76 m and a row length of 17 m.

Plots were subjected to chisel plowing in the fall of 2010 (Oct. 26) and moldboard plowing in the fall of 2011 (Nov. 09), followed by spring disking just before planting. The different fall tillage treatments were aimed at better residue incorporation. Although fall tillage can potentially affect N_2O emissions, we considered this effect negligible for growing season emissions as induced by spring fertilizer application. Corn (*Zea mays* L., var. Pioneer 38B14, 2700 Ontario Corn Heat Units, glyphosate tolerant) was planted on May 12 in 2011 and Apr. 27 in 2012 at 79 000 seeds ha^{-1}. Starter N fertilizer was applied as urea (30 kg N ha^{-1}) to all treatments at planting using the corn planter at 5 cm beside and below seeding depth. Fertilizer N rate treatments consisted of injecting urea-ammonium-nitrate (UAN) solution mid-row to a depth of 5–10 cm at rates of 0, 115 and 188 kg N ha^{-1} for total rates (including the starter N fertilizer) of 30, 145 and 218 kg N ha^{-1} using a UAN applicator equipped with 1.27-cm wavy coulters (Demco, Kent Farm Supply, 558 L). For the early application timing (at planting), UAN was injected mid-row on May 12 in 2011 and May 02 in 2012. The application at V8 occurred approximately 4–5 wk after planting on Jun. 15 in 2011 and Jun. 13 in 2012. Adequate P and K nutrition was maintained at the field site through application as a band application at seeding or as broadcast application prior to tillage, as needed. Herbicides Mesotrione (144 g ha^{-1}) and 2-chloro-4-ethylamino-6-isopropylamino-s-triazine (1 L ha^{-1}) were sprayed on 2011 May 21 and 2012 May 15 to control post-emergence weeds. Glyphosate was also applied on 2011 May 21 at 2.0 L ha^{-1}, and on 2012 Jun. 05 at 4.0 L ha^{-1}. In 2011 and 2012, the harvest of experimental units was completed on Nov. 01 and Oct. 12, respectively. In both years, whole plots were mechanically harvested using a four-row GleanerR42 rotary combine equipped with a GrainGage (HarvestMaster, Juniper Systems, Inc., Logan, UT 84321, USA) for determining plot grain weight, moisture, and test weight. Corn grain yield was adjusted at 15.5% moisture.

Gas Sampling and Flux Calculation

Nitrous oxide fluxes were measured approximately once per week for a total of 28 times in 2011 (from May 13 to Oct. 17) and 24 times in 2012 (from May 03 to Oct. 04) using non-flow-through non-steady-state (NFT-NSS) chambers (Rochette and Hutchinson 2005). Clear acrylic collars (0.6 m × 0.6 m; 0.15 m height; 6.35 mm wall thickness) were inserted to 10-cm depth into the soil between two rows of corn in the middle of each of 48 plots (12 treatments × 4 replicates) on May 12 in 2011 (0 d post-seeding) and May 02 in 2012 (5 d post-seeding). Collars did not cover the entire corn row width (0.76 m), hence, extrapolating the chamber measurements to the whole field may result in a slight overestimation of fluxes, as the areas not covered by the chambers were not affected by the N fertilizer application. However, this potential bias should be consistent for all treatments, and not affect comparison between treatments. Collars were left in the soil for the duration of the experiment, except for V8 N application treatments where collars were removed and reinstalled for the fertilizer application. Twenty-four acrylic chambers (0.6 m × 0.6 m; 0.15-m height) were used to enclose collars at each gas sampling event. Once chambers were placed on collars, four bricks were placed on top of the chamber to maintain the seal between the collar and the chamber. Gas samples were collected at 0, 10, 20 and 30 min after chambers were placed on the collars. Before each sample was collected, air was drawn from the chamber and released back into it to remove stagnant air in the sampling port (Rochette and Bertrand 2008). Air samples were drawn from the chamber sampling port with a 20-mL syringe (Becton-Dickinson, Franklin Lakes, NJ) and immediately injected into 12-mL pre-evacuated vials (Labco Exetainer, High Wycombe, UK) for analysis of N_2O concentration. Gas sampling events were conducted between 0900 and 1130 with the order of plot sampling randomized to remove any biases associated with changes in environmental conditions over this period. Samples were analyzed for N_2O concentrations using a gas chromatograph (Varian CP 3800; Varian Canada, Mississauga, ON) fitted with an electron capture detector at the Department of Soil Science at the University of Manitoba, Winnipeg, MB. The detection limit was 0.01 µL N_2O L^{-1} and the procedures followed are described in Tenuta et al. (2010).

Ancillary Measurements

One copper-constantan thermocouple was inserted at a soil depth of 5 cm beside each collar. Thermocouples were built according to the instructions of Berard and Thurtell (1990). On each gas sampling date, individual sensors were connected to a digital reader (Model HH23, OMEGA) and temperature values were recorded manually. Soil volumetric water content (0–12 cm depth) was measured at each sampling event using a portable time domain reflectometry (TDR) soil moisture meter (model TDR 300, Spectrum Technologies, Inc., Plainfield, IL) with a total of six measurements across each plot (two from each of three middle rows). Daily mean air temperature and barometric pressure were collected from the Elora Research Station weather station, located around 500 m from the experimental plots.

Soil bulk density was determined by the cylinder method (Blake and Hartge 1986). In 2011 before planting, eight undisturbed soil cores (5 cm diam. by 5 cm length) were collected from each of four blocks (5–10 cm depth). The soil bulk density average was 1.30 ± 0.03 Mg m^{-3}. Water-filled pore space was calculated as:

$$\%WFPS = (\text{volumetric moisture content} / \text{total soil porosity}) \times (100)$$

where soil porosity = 1 – (soil bulk density/soil particle density), with 2.65 Mg m^{-3} as the assumed particle density of soil (Linn and Doran 1984).

Inorganic N concentration (NO_3^--N and NH_4^+-N) were determined on soil samples taken at key corn stages to determine the effect of treatments on residual soil N. In 2011, samples were collected from the 0- to 30-cm depth on six dates: on May 12 (before N applied at planting), May 26 (at plant emergence), Jun. 14 (before N was applied at V8), Jul. 5 (at the 12th leaf stage) and Oct. 24 (at harvest). In 2012, samples were obtained on five dates: on May 01 (before N applied at planting), Jun. 08 (before N was applied at V8), Aug. 03 (at silking), Aug. 22 (at dough stage) and Sep. 25 (at physiological maturity). The 2012 Aug 03 sampling was limited to 0- to 15-cm depth because the soil was too dry and hard to sample at depth >15 cm. A soil core (2-cm diameter) of approximately 150 g was randomly collected from each of five locations between rows 2 to 5 of 6 using a soil corer. Samples were collected halfway between the corn row and the UAN band. Sample preparation and extraction were conducted as per procedure by (Maynard et al. 2008). The concentrations of NO_3^--N and NH_4^+-N in the extracts were determined spectrophotometrically using an auto analyzer (AACE 6.07 software, SEAL Analytical Inc., WI). Soil NO_3^--N was determined by the copper-cadmium reduction method, and NH_4^+-N was determined utilizing the Berthelot reaction (Searle 1984).

Data and Statistical Analyses

Soil N_2O flux was calculated using the following equation (Hutchinson and Livingston 1993):

$$FN_2O = (dN_2O/dt)(V/A)(M_{m.g}/V_m) \quad (1)$$

where dN_2O/dt is the rate of change of N_2O mixing ratio (mol mol^{-1} s^{-1}) inside the chamber during the time the chamber was placed on the collar, V is the chamber headspace volume (m^3), A is the soil surface area (0.35 m^2) covered by the collar, $M_{m.g}$ is the molecular mass of N_2O (44.01 g mol^{-1}), and V_m is the molar volume (m^3 mol^{-1}) inside the chamber, calculated according to the Perfect gas law:

$$V_m = RT/P \quad (2)$$

where R represents the universal gas constant (8.31 J K^{-1} mol^{-1}), T is the air temperature (K), and P is the barometric pressure (Pa).

The value of dN_2O/dt was calculated by linear or quadratic (using initial slope) regression of N_2O mixing ratio against time, whichever had the greater R^2 following procedure by Rochette and Hamel-Eriksen (2008) using an in-house program based on MATLAB® software version 8 (The MathWorks Inc., Natick, MA). The slope was tested for significance using a *t*-test, and dN_2O/dt was set to zero when it was not significantly different than zero. Cumulative N_2O emissions were calculated by the summation of daily estimates of N_2O flux obtained by linear interpolation between sampling dates, with an assumption that N_2O flux measured between 0900 and 1200 h was representative of the average daily N_2O flux.

Variance analyses, least square means and standard errors of estimates for cumulative N_2O emissions for the two experimental years were conducted using the PROC GLM procedure (SAS software version 9.1.3, SAS Institute, Inc., Cary, NC). The assumption for variance analysis was that treatment and other model effects were linear and additive and that the errors were random, independent and normally distributed about a zero mean with a common variance. Data set of cumulative N_2O emissions were tested for and found to pass the Shapiro-Wilk test (Bowley 2008). Variance analysis, least square means and standard errors for soil NO_3^--N and NH_4^+-N were performed using the PROC GLM procedure. A pair-wise comparison of all treatment means was conducted using Least Significant Difference (LSD) test. A stepwise regression analysis to examine the impact of N application rates, soil N concentrations, WFPS and soil temperature on cumulative N_2O emissions was performed using PROG REG procedure. The significance of the effects was determined using F-tests. All tests were conducted at a type 1 error probability of 0.05.

The fertilizer-induced emission factor expresses the difference between N_2O emissions from fertilized and non-fertilized plots scaled by the N application rate. The FIE was derived from linear or exponential regression curves of N_2O emissions as a function of N application rate, depending on the best fit, according to Kim et al. (2013). With a linear increase in N_2O emissions with N application rate, FIE is equal to the slope of the linear regression, while for exponential fits FIE = a(e^{bx} – 1)/x, where a and b are the exponential regression parameters and x is the N rate (Kim et al. 2013). We also compared our FIE values with estimates provided by the FIE model derived by Rochette et al. (2008a), which is based on a regional emission factor derived from experiments, scaled by the ratio of growing season (May–Oct.) precipitation to potential evapotranspiration (P/PE), that is, FIE = 0.022P/PE–0.0048.

RESULTS

Environmental Conditions

The mean air temperature was 16.9°C and mean soil temperature was 18.2°C for the growing season (May–Oct.) in 2011, and averaged 17.3 and 18.2°C, respectively, in 2012 (Fig. 1a, b). The growing season of 2011 (596 mm) was wetter than normal (492 mm), while 2012 (419 mm) was drier than normal (Table 1). There was 143 mm of precipitation during the month after N fertilizer application at planting in 2011, and 70 mm during the equivalent period in 2012 (Fig. 1c, d). In contrast, less rainfall was received during the month after side-dress application in 2011 (58 mm), a very dry period in 2012 (3 mm) (Fig. 1c, d). From August to October rainfall was above normal in both years, but drier in 2012 (Table 1). The WFPS was highest at the start and end of the growing season, averaging 74% in 2011 with only a short period with values < 60%, while the 2012 growing season mean was 45% and had close to 3 mo with < 60% values (Fig. 1e, f). During the 30 d following N fertilizer application at planting, WFPS was 85% in 2011 and 53% in 2012, and lower after application at V8 in both years, averaging 70 and 35% during 30 d, respectively (Fig. 1e, f).

Long-term and short-term history treatments did not have a significant effect on residual mineral N at the start of the growing season (before fertilizer application) in the 2 yr studied (data not shown). Higher soil NO_3^- content for the plots receiving N fertilizer at planting were observed for the Jun. 08 sampling in 2012 (Fig. 2b) just before the side-dress fertilizer was applied, but no significant differences were observed for any of the other sampling dates. Higher NH_4^+ content was observed for the plots receiving side-dress fertilizer at V8 for samples taken on 2011 Jul. 05 and 2012 Aug. 03 (Fig. 2c, d) only. Soil NO_3^- contents were highest for the largest N rate on two out of six samplings dates 2011 (Jul. 05 and Oct. 24) in 2011 and four out of five sampling dates (all dates except May 01) in 2012 (data not shown). Soil NH_4^+ content increased significantly with N rate in 2011 only on 2011 Jul. 05 and 2012 Jun. 08 (data not shown).

Nitrous Oxide Fluxes and Cumulative Emissions

Two distinct N_2O emission flux events were observed in 2011 associated with the interaction of N application rate with two timings of N application (Fig. 3a, c). Each event started approximately 1 wk after N fertilizer

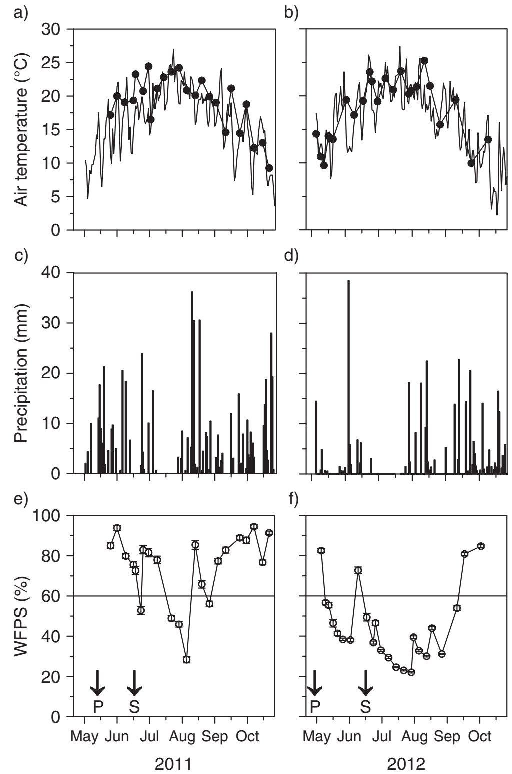

Fig. 1. Daily mean air temperature (line) and soil temperature at 5 cm measured at the time of gas sampling (solid circles) (a, b), precipitation (c, d) and water-filled pore space (WFPS) for the 0- to 12-cm layer measured at the time of gas sampling (e, f) during May to October at Elora, ON. Panels on the left are for 2011 and on the right for 2012. Arrows indicate timing of nitrogen fertilizer application at planting (P) and side-dress at corn 8th leaf stage (S).

application and lasted about 3 wk. The magnitude of N_2O fluxes was around 400% greater during the first event, associated with N applied at planting, compared with that during the second event, associated with N applied as side-dress at V8. Overall N_2O fluxes in 2012 were much lower, and the effect of N rate and timing was not as distinct as observed in 2012 (Fig. 3b, d).

Year of study had a significant effect on cumulative N_2O emission, and interactions between N application rate, timing and year were observed (data not shown). Hence, we present data for each year separately (Table 2). As history of N application did not have a significant effect on cumulative emissions, data have been pooled across these treatments. Timing and rate, as well as interaction between timing and rate of N application, had significant effects on cumulative N_2O emissions in

Table 1. Monthly precipitation (mm) for 2011 and 2012 and 30-yr average (1983–2012) at Elora, ON

Month	2011	2012	30-yr average[z]
		(mm)	
January	48	47	63
February	58	32	48
March	86	31	58
April	101	30	72
May	113	28	82
June	87	65	88
July	32	30	84
August	159	63	84
September	76	106	77
October	129	127	77
November	91	40	76
December	86	80	66
Annual total	1064	679	874

[z]30-yr average precipitation was calculated from monthly data obtained from Environment Canada for Elora Research Station, ON.

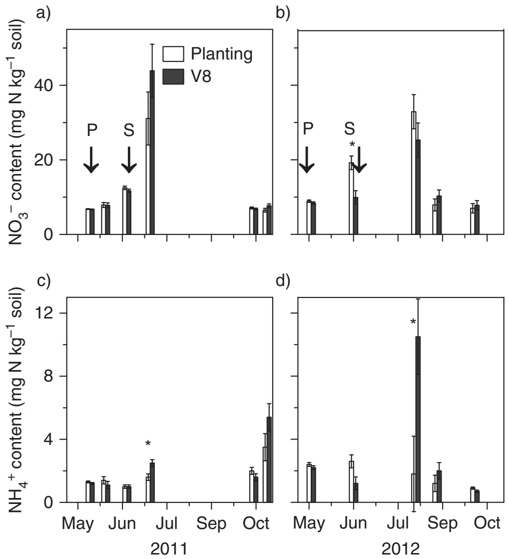

Fig. 2. Mean nitrate (a, b) and ammonium (c, d) content in soil samples taken during the growing season for treatments receiving nitrogen fertilizer at planting and as side-dress at the 8th leaf stage (V8) in corn in 2011 (left graphs) and 2012 (right graphs). Values were pooled across nitrogen rates and history treatments. Bars indicate standard error of mean and asterisk significant effects of timing on mineral N content.

2011 with higher emissions for treatments receiving the two highest N rates at planting compared to side-dress (Table 2). Averaged across all rate and history treatments, the cumulative N_2O emissions during the growing season in 2011 were significantly higher (140%) when N was applied at planting (2.12 kg N_2O-N ha^{-1})

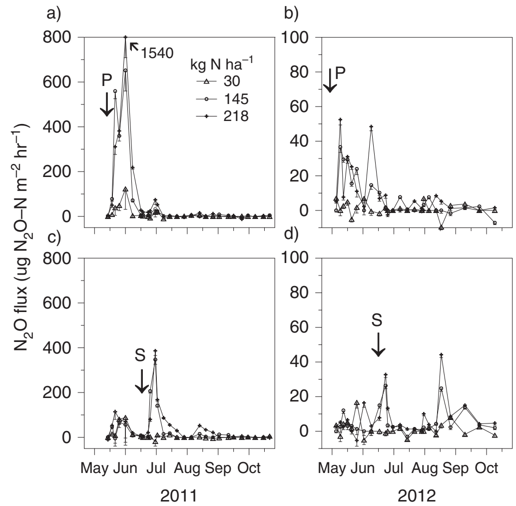

Fig. 3. Mean N_2O fluxes as affected by three rates of N applied at planting (a, b) and as side-dress (c, d) at the 8th leaf stage in corn on each of 28 sampling dates in 2011, and 24 sampling dates in 2012 at Elora, ON. Values for long-term and short-term history were pooled. Bars indicate standard error of mean. Note: the scale of graphs (a, c) is different than graphs (b, d).

compared with at V8 (0.88 kg N_2O-N ha^{-1}) (Table 2); 94 and 58% of the total growing season emissions occurred within 1 mo after N application at planting and V8, respectively. In contrast, only N rate had a significant effect on cumulative growing season N_2O emissions in 2012, and N_2O emission averaged 0.17 kg N ha^{-1} for both timings of N application (Table 2). Eighty percent of emissions in plots fertilized at planting occurred during the first month after N application in 2012, but for side-dress plots only 22% of total growing season emissions were observed during the equivalent period, with 61% occurring from August to October.

Grain Yield and N_2O Intensity

Corn grain yields were significantly affected by N application rates in both years, with the largest corn grain yield (10.8 and 7.43 Mg ha^{-1}, respectively for 2011 and 2012) associated with the highest N rate (218 kg N ha^{-1}) followed by lower grain yields with the two lower N rates (Table 2). Corn yields for the two highest N rates were not statistically different from one another in 2012, but both of them were significantly greater than the yield for the lowest N rate. Timing, rate and timing interaction did not affect corn grain yield (Table 2); neither did history of N application (data not shown).

Nitrous oxide intensity (emissions per kilogram grain yield) generally followed the same pattern as cumulative N_2O emissions showing significant effects for rate and timing of N application in 2011 and only rate effects in

Table 2. Growing season cumulative N_2O emissions, corn yield (adjusted to 15.5% moisture) and N_2O intensity as affected by history, rate, timing, and rate and timing interaction of N application[z] at Elora, ON, in 2011 and 2012. Other interactions were not significant and are not shown

	Cumulative N_2O emissions		Corn yield		N_2O intensity[y]	
	2011	2012	2011	2012	2011	2012
Treatments	(kg N_2O-N ha^{-1})		(Mg ha^{-1})		(g N_2O-N kg^{-1} grain)	
	N application rate effect					
30 kg N ha^{-1} (N_1)	0.318*c*	0.033*c*	6.29*c*	5.47*b*	0.051*c*	0.0048*c*
145 kg N ha^{-1} (N_2)	1.589*b*	0.195*b*	10.19*b*	7.11*a*	0.155*b*	0.0274*b*
218 kg N ha^{-1} (N_3)	2.604*a*	0.287*a*	10.82*a*	7.43*a*	0.241*a*	0.0391*a*
LSD (0.05)	0.89	0.073	0.455	0.67	0.08	0.011
SE	0.31	0.026	0.158	0.23	0.03	0.004
	Timing of N application effect					
Planting (T_1)	2.122*a*	0.163	9.09	6.76	0.208*a*	0.023
8th leaf stage (T_2)	0.884*b*	0.18	9.11	6.58	0.089*b*	0.025
LSD (0.05)	0.72	NS	NS	NS	0.07	NS
SE	0.25	0.021	0.129	0.19	0.02	0.003
	N application rate × timing of N application					
$N_1 \times T_1$	0.343*c*	0.021	6.12	5.48	0.056*c*	0.004
$N_1 \times T_2$	0.293*c*	0.046	6.46	5.47	0.045*c*	0.005
$N_2 \times T_1$	2.220*b*	0.191	10.3	7.36	0.217*b*	0.026
$N_2 \times T_2$	0.957*c*	0.199	10.09	6.86	0.094*c*	0.014
$N_3 \times T_1$	3.804*a*	0.278	10.87	7.43	0.353*a*	0.015
$N_3 \times T_2$	1.403*bc*	0.296	10.78	7.43	0.128*bc*	0.016
LSD (0.05)	1.25	NS	NS	NS	NS	NS
SE	0.44	0.04	0.22	0.33	0.04	0.01
	F value and probability level					
N application rate (N)	13.82**	25.04**	240.91**	20.07**	11.02**	19.53**
Timing of N application (T)	12.11**	0.33	0	0.42	12.87**	0.2
History of N application (H)	0.7	0.94	1.43	0.52	0.9	0.23
N × T	3.64*	0.03	0.83	0.37	3.49*	0.01

[z] For treatments, N at planting and at the 8th leaf stage, nitrogen was applied on May 12 and Jun. 15, respectively.
[y] N_2O intensity was calculated by dividing cumulative N_2O emissions (g N_2O-N ha^{-1}) of the crop period by corn grain yield (kg ha^{-1}).
a–c Means in the same column with different letters are statistically different at $P < 0.05$ according to LSD means comparison.
*, ** Significant at the 0.05 and 0.01 probability levels, respectively; NS, not significant.

2012 (Table 2). Significant reductions in N_2O intensity were observed at the lower N rate and later application timing.

DISCUSSION

In Ontario, soils are typically wet in the spring (April and May) as result of snow melt, ample rainfall and low evaporative water loss due to cool temperatures (Humaira et al. 2010). In contrast, warmer conditions in mid-summer (period following N applied at V8) are likely to result in more evapotranspiration leading to drier soils even if the same amount of precipitation is received as following N applied at planting. In 2011, greater cumulative N_2O emissions from N applied at planting were a result of higher soil moisture during the 4 wk post-N application when fertilizer-induced N_2O emissions were elevated (Figs. 1e, f and 3). Nitrous oxide emissions induced by fertilizer N application rise rapidly when WFPS exceeds 60% (Skiba and Ball 2002; Sehy et al. 2003). High WFPS leads to anoxic soil conditions, favourable for N_2O production through coupled nitrification-denitrification (Arah 1997), nitrifier denitrification (Wrage et al. 2001), and denitrification, the principal pathways resulting in N_2O emissions from agricultural soils (Robertson and Groffman 2007; Burton et al. 2008; Gillam et al. 2008; Zebarth et al. 2008). These conditions were present during much of the 2011 season, except for a 30-d period between Jul. 19 and Aug. 18 (Fig. 1b). Contrary to 2011, WFPS was mostly below 60% except at the beginning and very end of the growing season in 2012 and the seasonal mean WFPS (46%) was significantly lower than that of 2011 (73%) (Fig. 1e, f). Indeed, WFPS was the main variable affecting N_2O growing season emissions over the 2 yr (Fig. 4), while N rate explained part of the variation (partial $r^2 = 0.11$) and soil mineral N did not explain any of the variation in N_2O emission observed (data not shown).

The effect of interaction between rate and timing of N application on cumulative N_2O emissions in 2011 was significant (Table 2), and the response of emissions to N rate was exponential and distinct between the two timings (Fig. 5a). However, the interaction between N rates and timing of application on cumulative N_2O emissions was not significant in 2012, with similar slopes for both linear responses (Fig. 4b). The non-linear response in 2011 agrees with recent suggestions of FIE

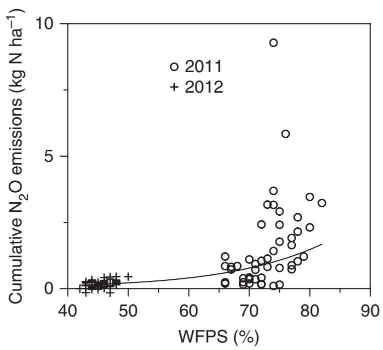

Fig. 4. Change in cumulative N_2O emission as a function of mean water-filled pore space for 2011 and 2012. Values shown are for individual replicates (four) for each studied treatment (3 rates × 2 timings × 2 histories). The line shows an exponential fit ($Y = 0.00842e^{(0.0646X)}$) with $r^2 = 0.51$ and $n = 89$ (<96 potential observations due to some negative emissions).

factors that increase with high N rates (> 150–200 kg N ha^{-1}) (Hoben et al. 2011; Kim et al. 2013), although additional rates would have been beneficial for a better curve fitting of our data. Nevertheless, our data clearly show how the N_2O emission response to N rates can be mediated by timing of application and soil conditions. Although the total precipitation in May–June 2011 (200 mm) was above normal, the frequency of occurrence (a higher amount occurs with a frequency of 10 times in 30 yr) indicates results obtained in this year are representative of Ontario conditions. In contrast, rainfall lower than observed May–June 2012 (93 mm) occurs with a frequency of only four times in 30 yr, suggesting 2012

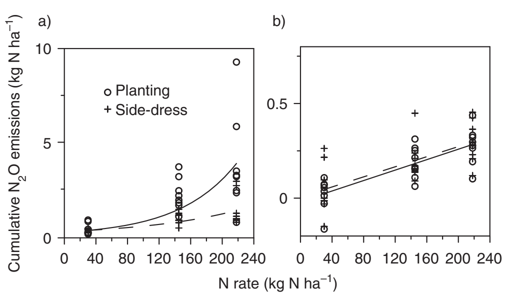

Fig. 5. Relationship between cumulative N_2O emissions and N application rate in (a) 2011 and (b) 2012 under two timings of N application (at planting and as side-dress at the 8th leaf stage) at Elora, ON. Lines in (a) show exponential fits (solid line: $Y = 0.194e^{(0.0137X)}$, $r^2 = 0.71$ and $n = 24$; dashed line: $Y = 0.234e^{(0.00808X)}$, $r^2 = 0.685$ and $n = 24$) and (b) shows linear fits (solid line: $Y = 0.00138X - 0.0175$, $r^2 = 0.616$ and $n = 24$; dashed line: $Y = 0.00133X + 0.0058$, $r^2 = 0.454$ and $n = 24$) Values for short- and long-term histories were pooled. Note different Y-axis scale in two graphs.

results are not as likely. Hence, timing of N applications can be used as a tool to mitigate N_2O emissions during most years.

To compare our growing season (May to October) FIE to the factors estimated using the Rochette et al. (2008a) approach for Ontario, we derived average emission factors across N rates, timings and histories of N application using linear regressions for 2011 (1.21%) and 2012 (0.14%). The estimated FIE, based on the ratio of measured precipitation to estimated potential evapotranspiration (P/PE) over May to October was similar to observed values in 2011 (1.28%), but higher than observed in 2012 (1.46%). Although 2012 was very dry from May to August, above-normal rainfall in September to October yielded a higher P/PE value compared with 2011. This meant the timing of rainfall was a factor resulting in a higher estimated FIE factor for 2011 than observed. In addition, timing of N application also affected FIE with a significantly higher value for N applied at planting (1.82%) compared with at V8 (0.59%) in 2012, both values derived using a linear regression approach. If the apparent exponential nature of the response is considered (Fig. 4) then contrasting values are obtained for the recommended N rate (145 kg N ha^{-1}) applied at planting (0.84%) versus as side-dress (0.36%), as well as the highest N rate studied for these two timings (1.67 vs. 0.52%, respectively). This emphasizes the need to consider non-linear effects and N management practices when deriving FIE.

Since timing of N application did not influence corn grain yield, N_2O intensity followed the same patterns as N_2O emissions per unit land area in both years. However, the dry conditions in 2012 reduced N_2O emissions and N_2O intensity by a greater percentage (89 and 84%, respectively) than the reduction in corn yield (27%). In previous studies, depending on the N application rates, the N_2O intensity varied from 0.009 to 0.697 g N_2O-N kg^{-1} grain (Weiske et al. 2001; Kaharabata et al. 2003; McSwiney et al. 2005; Almaraz et al. 2009), which is generally higher than the values obtained in this experiment (0.051 to 0.241 g N_2O-N kg^{-1} grain in 2011, and 0.005 to 0.391 g N_2O-N kg^{-1} grain in 2012). The overall N_2O intensity in 2012 was very low due to low WFPS as discussed above. This result is consistent with previous knowledge that N_2O fluxes are dependent on the interaction of environment and management (Smith et al. 2003).

The lack of an effect of history (short- and long-term) of N application on N_2O emissions in either 2011 or 2012 is likely related to similar soil NO_3^- and NH_4^+ content associated with the two histories of N application. Although, for the long-term history, the low and high N rate treatments had received the same low and high rates continuously for 4 yr this was not reflected in soil NO_3^- or NH_4^+ content. Thus, the current rate of N application appears to exert a predominant influence on soil NO_3^- or NH_4^+ content rather than the rate in the previous years, and in terms of soil N_2O emissions, soil

WFPS and N rate were the most important contributing factors. In the humid environment of Ontario, N in the soil may be too mobile to exert any residual effect in subsequent years. Liang et al. (1991) reported that most of the residual NO_3^--N from soil can be lost if the non-growing season precipitation is very high. It is also important to consider that nitrous oxide emissions can be significant during the non-growing season. Wagner-Riddle et al. (2007) have shown that a large fraction of annual emissions ($>50\%$) occur over the Nov. to Apr. period at this site, and non-growing season emissions are larger for soils with high fall nitrate (Wagner-Riddle and Thurtell 1998). Limiting measurements to the growing season misses these emissions and results in underestimation of total annual emissions, so these should be used with caution. However, comparison between treatment effects over the growing season is still valid. Indirect N_2O emissions associated with leaching of residual N in the non-growing season should also be considered but were beyond the scope of this study. For example, in a dry year increased residual nitrate in the fall associated with lower plant N uptake could lead to nitrate leaching losses (Hernandez-Ramirez et al. 2011), but similar fall nitrate levels in the 2 yr suggest this was not the case in this study.

CONCLUSIONS

In a typical wet Ontario spring, N applied as side-dress at V8 in corn compared with at planting resulted in a significant reduction in N_2O emissions at the recommended (145 kg N ha^{-1}) and above recommended (218 kg N ha^{-1}) N application rates. Corn grain yield was not affected by timing and, as a result, N_2O emission intensity was also significantly reduced when N fertilizer application was timed to better coincide with plant N uptake. Water-filled pore space was the main controlling factor on N_2O growing season emissions; hence, a large reduction in emissions can be expected in years when WFPS is lower following side-dress compared with following planting. Lower WFPS in June is usually present in Ontario due to higher evapotranspiration as induced by higher temperature and plant water uptake, and hence we suggest this management practice has potential for long-term N_2O emission reduction. Future studies could investigate whether different forms of N (UAN vs. UAN plus urease or nitrification inhibitors) have a confounding effect on N_2O emissions when applied at planting or as side-dress at V8. The need for mitigation options for reduced N_2O emissions under the humid Ontario conditions will become more pressing in the future as long-term climate forecasts for North America point to the possibility of increased rainfall and in particular, increased frequency and intensity of rainstorm events (IPCC SREX 2012). Management of application timing to avoid periods with high WFPS ($>60\%$) could contribute to reduction of N_2O emissions.

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