



Low Nitrous Oxide Emissions in a Boreal Spruce Forest Soil, Despite Long-Term Fertilization

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Nitrogen (N) fertilization can increase stem wood production by several hundred percent in boreal forests. At the same time, there are concerns about the environmental consequences of N fertilization, especially considering losses of the greenhouse gas nitrous oxide (N₂O) to the atmosphere. Soils are a large contributor to N₂O emissions on a global scale. The aim of this study was to investigate the consequences of long-term nutrient optimization fertilization on N₂O emissions in a boreal forest in Northern Sweden. Field N₂O flux measurements were conducted during 2 years with manual and automatic chambers, as well as gas probes in the snow. The N₂O emissions were generally low during the whole period of measurements, both from the control and fertilized plots. The emissions were generally highest during the winters, as well as the variability in the observed values. Overall, N₂O emissions from fertilized plots were about twice the control, which could be explained by changes in the soil carbon-to-nitrogen ratio.

Keywords: forest fertilization, nitrous oxide emissions, boreal forest soil, Norway spruce, carbon-to-nitrogen ratio

INTRODUCTION

Nitrogen (N) is the main limiting nutrient for growth in many boreal forests (Tamm, 1991; Högberg et al., 2017) and fertilization is therefore an effective way to increase the net primary production in this slow growing ecosystem (Axelsson and Axelsson, 1986; Linder, 1987). In Northern Sweden, fertilization can increase stem wood production with more than 350% (Bergh et al., 2005). However, there are concerns about the environmental consequences of intensive forest fertilization practices, primarily due to the drastic changes induced on the ecosystem level. Sustained N addition to initial N-limited ecosystems can in the long-term lead to an N saturation of the ecosystem (Aber et al., 1998), which increases the risk of N losses to water and air (Bremner and Blackmer, 1978; Binkley et al., 1999). Nitrous oxide (N₂O) emissions from the forest floor have been shown to be related to N input (Zechmeister-Boltenstern et al., 2002) and to the mineral N content in the soil in general, especially in soils where the dominant form of N is nitrate (NO₃⁻) (Davidson et al., 2000). Soils are, on global scale, a source of N₂O to the atmosphere (Ciais et al., 2013) and forests contribute to a significant part of the emissions (Bowden et al., 1990; Ambus and Christensen, 1995; Kesik et al., 2005). Leaching of NO₃⁻ is often related to anthropogenic inputs, either N deposition or fertilization (Sponseller et al., 2014). In non-fertilized boreal forests with low N deposition, the majority of the leaching to streams and waters is in the form of dissolved organic nitrogen (Hedin et al., 1995; Stepanauskas et al., 2000).

Generally, forest N fertilization enhances N₂O emissions, although a few exceptions exist (Gundersen et al., 2012; Shrestha et al., 2015). There are, though, few data from boreal forests, which are usually characterized by low N₂O emissions (Bai et al., 2012), due to its N-limited status. Therefore, N fertilization of this ecosystem can be expected to lead to increased N₂O emissions. Indeed, in the review by Gundersen et al. (2012) the largest stimulation of N₂O emission by N addition was observed for a wet hemiboreal forest in south-western Sweden, confirming earlier results from the same forest (Klemetsson et al., 1997). In contrast, Maljanen et al. (2006) did not observe stimulated N₂O emission in a boreal forest in southern Finland. The paucity of N₂O emission from N-fertilized boreal forests and the contradicting results so far, motivated us to conduct N₂O measurements from a N-limited boreal forest in Northern Sweden (Flakaliden) that had been subjected to fertilization (optimal nutrient management, including addition of 50–100 kg N ha⁻¹ year⁻¹) for more than 20 years (Linder, 1995). The leaching of inorganic N from the control and fertilized plots in this forest is less than 1 kg N ha⁻¹ year⁻¹ (Andersson et al., 2002), but so far N₂O emissions were unknown. We expected that the unfertilized forest is characterized by low N₂O emissions, as generally found for N-limited boreal forests, and that the long-term fertilization led to enhanced N₂O emissions, particularly under wet condition. We tested this with field N₂O flux measurements during two full years.

MATERIALS AND METHODS

Site

Flakaliden (64°07'N, 19°24'E) is a Norway spruce [*Picea abies* (L.) Karst.] forest situated in Vindeln municipality, Northeast Sweden. The altitude is 310–320 m above sea level. The climate is boreal, with short, cold summers with long days and long winters with short days. Mean annual temperature (MAT) and precipitation (MAP) for the period 1990–2019 at the site is 2.4°C and 647 mm, respectively (Linder, pers. comm.). One-third of the precipitation falls as snow (Bergh et al., 1999). The annual N deposition in the area is 4.6 kg ha⁻¹ year⁻¹ (Hedwall et al., 2013). The soil texture is sandy and soil type Podzol, with an organic layer between 3 and 6 cm. In 1963, the site was planted with seedlings of Norway spruce of local descent. In 1986, an experiment with different types of fertilization treatments was started (Linder, 1995). In this study, we investigated plots that were annually given a solid fertilizer mix (nutrient optimization) containing among other nutrients an amount of 50–100 kg N ha⁻¹ in the form of ammonium nitrate (NH₄NO₃, hereafter referred to as fertilized plots), as well as untreated control plots. In the years of N₂O measurements, N was added each year in May at a rate of 50 kg N ha⁻¹. The field layer in the fertilized plots was very sparse, while in the control plots it was dominated by dwarf-shrubs (Strengbom et al., 2011).

Field Measurements of N₂O

Emissions of N₂O were measured during two full years from February 2009 to April 2011 using three different methods:

manual chambers in June–December 2009, automatic chambers June–December 2010, and with snow probes during winters (March–April 2009, March–May 2010, and January–April 2011). The study was planned for automatic chambers, which though were delayed and could not start before year 2. The automatic chambers set a limit to the distance between the chambers, due to the maximum length of the tunings between the chambers and analyzer. Therefore, only one fertilized plot and one control plot (50 × 50 m, each) could be measured. In each plot, six metal collars (45 × 45 cm) collars were inserted permanently into the ground to a depth of 2 cm to minimize disturbance during measurements. As previous research found that the spatial dependency of N₂O emission is in the range of <1 to ~70 m (Ambus and Christensen, 1994; Velthof et al., 1996; Turner et al., 2008), our approach likely covered most of the spatial variability in at the study site.

Measurements with manual chambers ($n = 6$ per treatment) were done weekly in the snow free period 2009 (June–December). The chambers were closed with a lid on top of the permanently installed metal frames. The inside of the lids were lined with a neoprene rubber sealing to avoid air leakage when closed. The volume of each chamber was measured individually, due to differences in topography between the collars. During measurements, gas samples were taken every 10 min during 30 min (four samples), starting 5 min after chamber closure (total chamber closure was 35 min) following the procedure described in Klemetsson et al. (1997). The sample vials were analyzed on a gas chromatograph and the fluxes were calculated with linear regression, from the concentration changes in the chambers during closure (Klemetsson et al., 1997).

In June to December 2010, three automatic chambers per treatment were used, on the same collars as for the manual chamber measurements. The chambers, were moved every week between the six collars to avoid long-term chamber effects (e.g., moisture and temperature). The chambers had a lid and sides of transparent polythene plastic, held together by an aluminum frame. The volume of the chamber (including the collar) was about 0.24 m³. The gas sampling system consisted of a tunable diode laser (Campbell Scientific, TGA100A), a valve system, and a data logger (Campbell Scientific, CR-3000). The data logger controlled pressure, flow, opening, and closure of the chambers, which chamber that was connected to the laser, as well as data collection. The chambers were closed for 40 min/h, and sampling air from each chamber was measured for 20 s every other minute. The first 10 s of the measurements were discarded due to remaining air from the previous sample, and the remaining 10 s were used for calculations of concentrations. The concentration increase in the chambers was calculated with linear regression. For more information about the automatic chamber system, see Hedenrud (2015).

During the winter, the concentrations of N₂O in the snow profile were measured with gas probes, which were conducted in the near vicinity (0.5–1 m) of the chamber frames, to avoid disturbance of the abiotic conditions underneath the chambers, but representing the same conditions. The gas samples from the probe were transferred into gas vials sealed with a butyl membrane and analyzed using gas chromatography. Snow depth

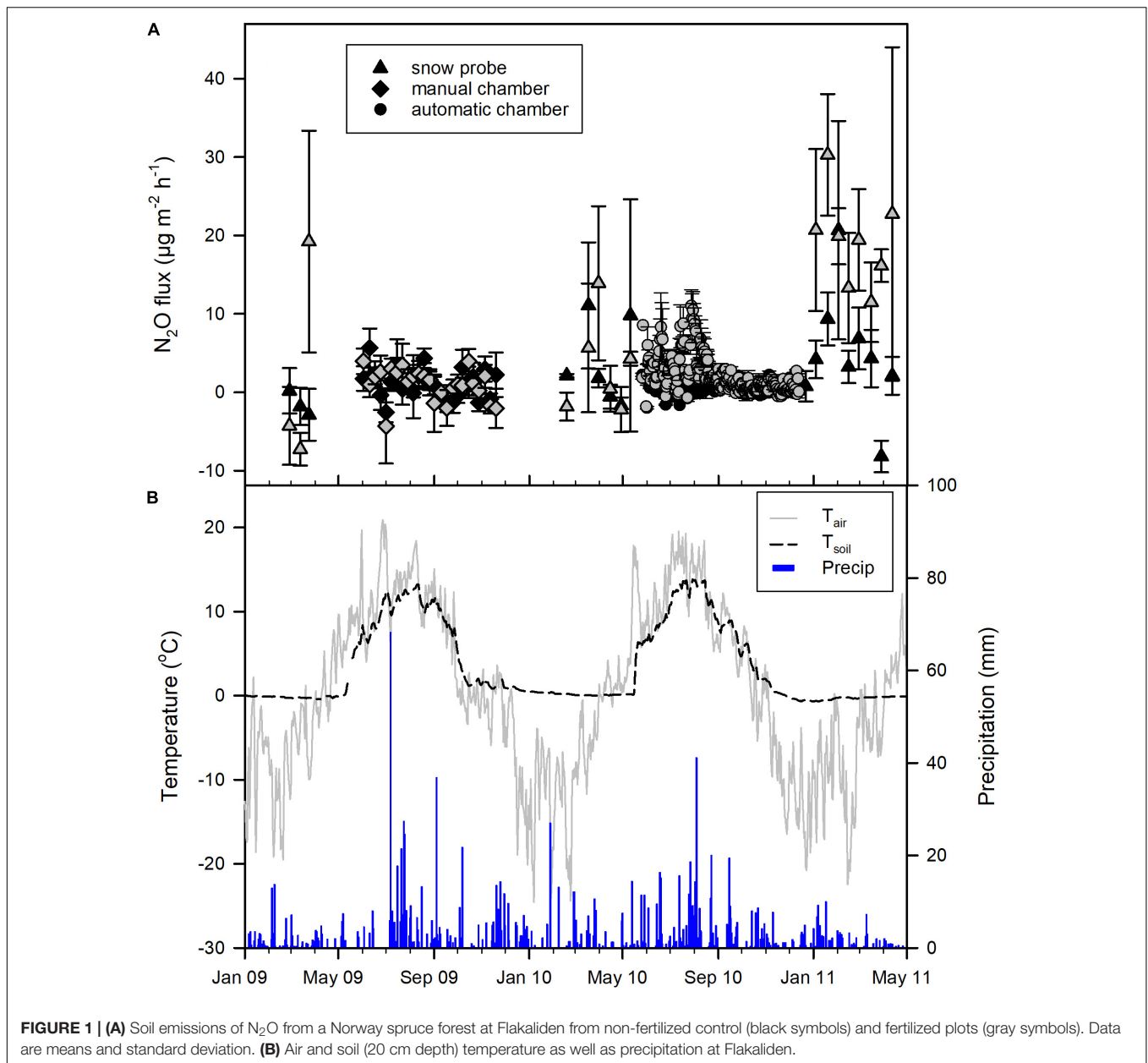


FIGURE 1 | (A) Soil emissions of N₂O from a Norway spruce forest at Flakaliden from non-fertilized control (black symbols) and fertilized plots (gray symbols). Data are means and standard deviation. **(B)** Air and soil (20 cm depth) temperature as well as precipitation at Flakaliden.

and density were measured at each sampling occasion. The net fluxes of N₂O were calculated according to Sommerfeld et al. (1993). The snow probe method is described in detail by Björkman et al. (2010). In 2009, the snow probes could not be used when the snow layer became too thin. New probes were used in 2010, which were adapted to lower snow depths. Björkman et al. (2010) reported that soil respiration measured with snow probes was not different from fluxes measured with chambers on top of the snow. In addition, data from snow probes had lower variability.

Statistics

Flux data were analyzed with the non-parametric Wilcoxon Signed Ranks test for each measured period separately, since the

data was obtained with different measurement techniques and with different frequency. Statistical analyses were performed with SPSS (IBM SPSS Statistics 25). In addition, Spearman Rank Order Correlation was conducted using SigmaPlot (version 14.0; Systat Software, Inc.).

RESULTS

The years 2009 and 2010 were rather normal in terms of precipitation (MAP of 673 and 618 mm, respectively; **Figure 1B**). While 2010 was colder (MAT of 0.5°C) than the long-term average, 2009 was rather normal warm (MAT of 2.0°C; **Figure 1B**).

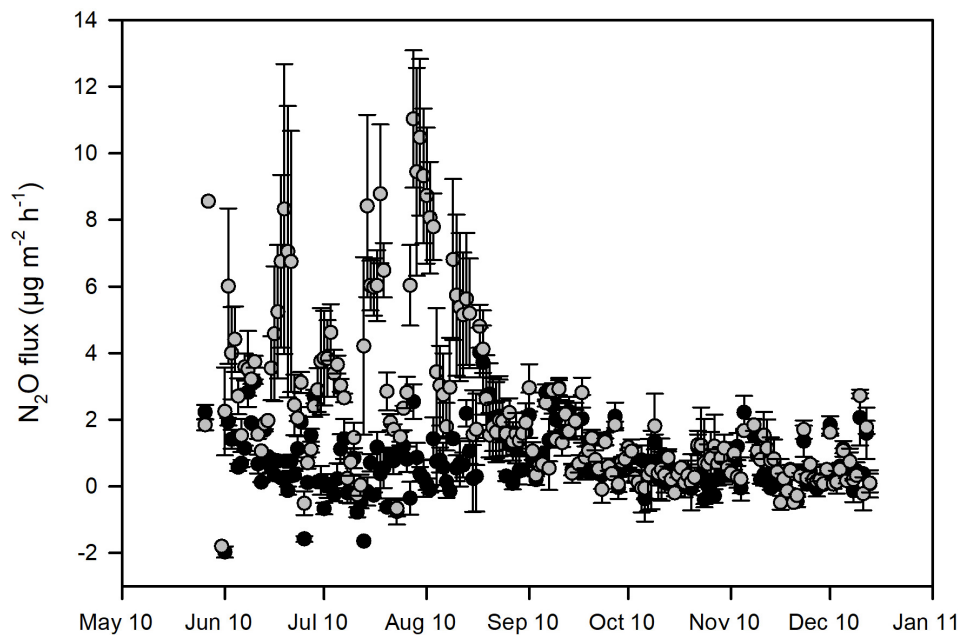


FIGURE 2 | Detail of the soil N₂O emissions from a Norway spruce forest at Flakaliden measured with automatic chambers at daily resolution during the summer and fall 2010, from non-fertilized control (black symbols) and fertilized plots (gray symbols).

The N₂O emissions were low from both the control and fertilized plot at the site. The mean (\pm SD) N₂O flux for the entire measurement period was 0.97 (\pm 2.15) $\mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$ for the control plot and 2.67 (\pm 4.30) $\mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$ for the fertilized plot (Figure 1A). During the first year of measurements (March 2009–February 2010) there was no statistical significant difference between control and fertilized plots, but during summer and fall 2010, when fluxes were measured with automatic chambers, the emissions from the fertilized plot were significantly higher compared to the control plot ($p < 0.001$, see Figure 2). Fertilization was conducted at the end of May 2010 and the significantly higher fluxes were primarily detected from June to September. During the rest of the period measured with automatic chambers (October–December) the emissions were low and there was no significant difference between control and fertilized plots (Figure 2). When measurements were conducted with snow probes, the emissions were both high and had high variability (Figure 1A). Winter measurements showed significant differences between the control and the fertilized plot only in 2011 (January–April), where the fertilized plot had higher emissions compared to the control (Figure 1A).

The mean of the emissions is biased by sampling frequency, due to the use of manual and automatic chambers during years 1 and 2, respectively. We therefore in addition integrated fluxes by linear interpolation between subsequent measurement days, but not for gaps between chamber and snow probe measurements. The mean of these integrated fluxes was 1.98 and 4.79 $\mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$ for control and fertilized plot, respectively, hence about twice the average daily fluxes. This difference is due to the less frequent measurements of winter emission, which, though, often were higher than summer and fall emissions (Figure 1A).

Correlations with climatic variables were conducted for the period of N₂O flux measurements by automatic chambers and for the period with all chamber measurements (manual and automatic). While no significant correlations were detected for the fluxes from the control plot (Table 1), N₂O emissions from the fertilized plot was positively correlated with both, soil and air temperature, explaining 34–41% of the observed variability (Table 1). In addition, the correlation with precipitation was significant for the fertilized plot, but with much lower explained variability (2–3%; Table 1).

DISCUSSION

The N₂O emissions were mostly low during the whole series of measurements, both from the control and fertilized plots,

TABLE 1 | Spearman Rank Order Correlation coefficient between N₂O fluxes and climatic factors: air and soil (20 cm depth) temperatures as well as precipitation for the period with automatic chambers and period including all chamber measurements (manual and automatic).

| | Temperature air | Precipitation | Temperature soil |
|-------------------------|-----------------|--------------------|------------------|
| Control plots | | | |
| Automatic | 0.105 | −0.083 | 0.114 |
| All chambers | 0.078 | −0.056 | 0.092 |
| Fertilized plots | | | |
| Automatic | 0.632* | 0.176 ⁰ | 0.639* |
| All chambers | 0.584* | 0.153 ⁰ | 0.591* |

⁰ $p < 0.05$; * $p < 0.01$.

which is a general observation for boreal forests (Klemmedtsson et al., 1997; Kim and Tanaka, 2003; Matson et al., 2009; Ullah et al., 2009). The N₂O emissions were highest during the winter, which has been reported before for forest ecosystems (Sommerfeld et al., 1993; Brooks et al., 1997; Groffman et al., 2006). Snow insulates the ground, which means that the soil temperature can be above zero degrees although the air temperature is lower, allowing N₂O production to continue under the snow (Enanga et al., 2016). The large variability in the winter measurements is connected to the sampling method with gas probes, where the variation between the different probes was much higher than for the chambers. This may be due to variations in snow density, which strongly affects the emissions from the snow (Björkman et al., 2010).

Overall, the N₂O emissions from the fertilized plot was about twice the control, but statistical significance was only observed during the second year, when measurements were conducted with automatic chambers. It is a common observation that N₂O fluxes have high temporal variability (Groffman et al., 2009) and with more frequent sampling, there is a better chance of capturing this variability. Indeed, a recent study concluded that at least two flux measurements per day are required to achieve robust annual emission estimates and to detect differences between treatments in field trials (Lammirato et al., unpublished data). We therefore can conclude with confidence that fertilization of the boreal forest at Flakaliden did enhance N₂O emissions. While the measurements were conducted in a nutrient optimization experiment, we assign most of the enhanced N₂O emission to N addition, as it is well known that N is the main limiting nutrient for growth in boreal forests (Tamm, 1991; Högberg et al., 2017). We, though, cannot fully rule out that other nutrients might have stimulated microbial growth and, potentially, N₂O production and emission.

Two review papers (Gundersen et al., 2012; Shrestha et al., 2015) concluded that N fertilization generally enhances N₂O emission from forests, although exceptions exist. However, few of the reviewed studies have been conducted in boreal forest. While Maljanen et al. (2006) did not observe enhanced N₂O emission due to fertilization in a boreal forest in Finland, N fertilization turned a hemi-boreal forest in south-western Sweden from a sink into a significant source of N₂O [reported in Gundersen et al. (2012)]. Large stimulation of N₂O emission by fertilization was also reported for a subalpine coniferous forest after 8 years of yearly N addition (Li et al., 2019).

The difference between fertilized and control fluxes in our study can largely be explained by the C/N ratio of the soil, which is a good proxy for N₂O emissions from forest soils (Klemmedtsson et al., 2005; Pilegaard et al., 2006). Björnsne (2018) measured several soil properties in the investigated forests plots in 2016 and

found significantly lower C/N ratio in the fertilized than control plots (26.4 and 32.2, respectively). In addition, other changes in soil properties could have contributed to the higher N₂O fluxes from fertilized plots. Björnsne (2018) also observed numerically lower soil pH in the fertilized plots, which could lead to higher N₂O emissions as well (Weslien et al., 2009).

Earlier studies have estimated N₂O emissions derived from fertilization in forests to be 0.5–1% of the added N (Macdonald et al., 1997; Papen and Butterbach-Bahl, 1999; Maljanen et al., 2006). The comparable number for the present study is 0.3%, based on the integrated fluxes over the entire measurement period. In a pan-European study (Gundersen et al., 2012) the increase in mean N₂O emissions due to fertilization ranges from 2 to 230 mg N m⁻² year⁻¹, and our study would range at the lower end (9 mg N m⁻² year⁻¹). Overall, we conclude that N is even in the fertilized plot relatively tightly cycled and efficiently used, pointing to prevailing N limitation even after long-term N fertilization.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

LK designed the experiment. PW conducted measurements and flux analysis. All authors discussed and interpreted results. A-KB and TR drafted the manuscript, to which all authors contributed.

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