Effects of nitrogen fertilization on the fluxes of NO, CH₄, and CO₂ from soils in a Florida slash pine plantation

MARK S. CAFFEO, WILLIAM T. PETERSON, JERRY M. MILLO, and PAUL A. STEUDLER
The Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA 02543, U.S.A.

HENRY L. GHOZL
Department of Forestry, University of Florida, Gainesville, FL 32611, U.S.A.

DAVID LEWIS
United States Environmental Protection Agency, Environmental Research Laboratory, Athens, GA 30613, U.S.A.

Received January 12, 1993
Accepted June 9, 1993


We measured fluxes of NO, CH₄, and CO₂ from control and urea-nitrogen fertilized soils of a mature slash pine (Pinus elliottii var. elliottii Englem.) plantation in Alachua County, Florida. The fertilization did not affect CO₂ emissions, but significantly increased the emissions of NO and lowered the uptake of ammonia CH₄. Daily average NO emissions from the fertilized soils were 8–600 times higher (12–74 μg N·NO·m⁻²·h⁻¹) than daily average NO emissions from control soils (0.02–0.4 μg N·NO·m⁻²·h⁻¹). Daily average CH₄ uptake by the fertilized soils were 5–20 times lower (0.001–0.007 mg CH₄·C·m⁻²·h⁻¹) than daily average CH₄ uptake by control soils (0.013–0.035 mg CH₄·C·m⁻²·h⁻¹). We also measured the relative activities of the bacteria populations that were responsible for CH₄ oxidation in the control and fertilized soils. Results from these measurements suggest that fertilization shifted the relative activities of the CH₄ oxidizing bacteria from those dominated by methanotrophs in the control soils to those dominated by nitriﬁying bacteria in the surface (0–2 cm) of the fertilized soils. The shift in relative activities of these bacteria may have been responsible for the lower CH₄ uptake by the fertilized soils.


Les flux de NO₂, CH₄ et CO₂ ont été mesurés dans des sols témoin et des sols fertilisés en azote urée dans une plantation naturelle de pin de Floride (Pinus elliottii var. elliottii Englem.) du Comté d’Alachua, en Floride. La fertilisation n’a pas affecté les émissions de CO₂ mais a significativement augmenté les émissions de NO et a diminué le prélèvement de CH₄ atmosphérique. Les émissions moyennes journalières de NO des sols fertilisés ont été de 8 à 600 fois plus élevées (12–74 μg N·NO·m⁻²·h⁻¹) que les émissions moyennes journalières des sols témoin (0.02–0.4 μg N·NO·m⁻²·h⁻¹). Le prélèvement moyen journalier de CH₄ par les sols fertilisés a été de 5 à 20 fois plus faible (0.001–0.007 mg C·CH₄·m⁻²·h⁻¹) que le prélèvement moyen journalier de CH₄ par les sols témoin (0.013–0.035 mg C·CH₄·m⁻²·h⁻¹). Les activités relatives des populations de bactéries responsables de l’oxydation de CH₄ ont aussi été mesurées dans les sols témoin et les sols fertilisés. Les résultats suggèrent que la fertilisation a déplacé les activités relatives des bactéries oxidant le CH₄ de celles dommées par les méthanotrophes dans les sols témoin à celles dommées par les nitriﬁantes dans les deux premiers centimètres des sols fertilisés. Le déplacement dans les activités relatives de ces bactéries peut avoir été responsable des plus faibles prélèvements de CH₄ par les sols fertilisés comparés aux sols témoin.

Introduction

Over the past 40 years, atmospheric concentrations of nitrous oxide (N₂O), methane (CH₄), and carbon dioxide (CO₂) have increased and continue to increase at annual rates of 0.3, 0.6, and 0.9%, respectively (Watson et al. 1992). These increases are of concern because these gases have the potential to affect the earth’s climate and atmospheric chemistry.

The increasing atmospheric concentrations of N₂O, CH₄, and CO₂ have led to studies of the factors controlling natural sources and sinks of these gases. Results from these studies suggest that N fertilization of soils in many ecosystems increased N₂O emissions into the atmosphere (Dusbury and M-Connaughtey 1986; Hutchinson and Mosier 1979; Keller 1987; et al. 1983, 1988, 1990; McKenney et al. 1980; Mosier et al. 1991). Yield studies in temperate grasslands and forests demonstrated that N fertilization lowers CH₄ uptake by the fertilized soils (Mosier et al. 1991; Steudler et al. 1989).

Additional ecosystem studies are needed to determine if the N induced suppression of CH₄ uptake is a widespread phenomenon and to better understand the mechanisms responsible for the N induced suppression of CH₄ uptake. The effect of N fertilization on CO₂ emissions (from forest soils is not clear; some studies report no changes (Repoveetskaya 1967), others report increases and decreases in CO₂ emissions (Bromme and Beuse 1992; Silvola et al. 1985). Collectively, results from these studies suggest that the exchange of N₂O, CH₄, and CO₂ between the atmosphere and soils in many ecosystems is affected by N fertilization.

Nitrogen fertilization is common practice in commercial forestry in the southeastern United States. Most of the past research associated with fertilized pine plantations focused

1Author to whom all correspondence should be addressed.
2Present address: Department of Biology, West Virginia University, Morgantown, WV 26505, U.S.A.
on stand growth, productivity, and time to harvest (Allen et al. 1990; Colbert et al. 1990; Jokela et al. 1990; Stone 1983). The effects of $N$ fertilization on the fluxes of $N_2O$, $CH_4$, and $CO_2$ from soils of these fertilized pine plantations are not well known. In this paper, we describe the effects of 4 years of urea-$N$ fertilization on the exchange of $N_2O$, $CH_4$, and $CO_2$ between the atmosphere and soils of a mature slash pine (Pinus elliottii var. elliottii Engelm.) plantation in Florida.

**Study site**

The study site was a 60-ha block of a slash pine plantation located approximately 20 km northeast of Gainesville in Alachua County, Florida (29°N, 82°W). The overstory was dominated by eastern white pine (Pinus taeda) and slash pines that were 26 years old in 1991. The understory was dominated by saw palmetto (Serenoa repens) and a sparse cover of grasses and forbs overlying dead pine needle. The soils (Ultic Hapludolls) are sandy, poorly drained, and have low organic matter and low nutrients (Chelot et al. 1985). The groundwater table fluctuates between the surface and approximately 2 m below the surface throughout the year. Average annual precipitation and temperature (1955–1987) are 1342 mm and 21.7°C, respectively (National Oceanic and Atmospheric Administration 1989).

In 1986, the study site was divided into sixteen 50 × 50 m plots, with at least 100 m separating the plots (Gholt et al. 1991). From February 1987 through December 1991, eight plots were fertilized quarterly with a complete (N-P-K) fertilizer. Microorganisms (C, Bo, Fe, Mn, Zn, and Mo) were added to the fertilized plots in 1986. Nitrogen as urea was added at an annual rate of 180 kg N/ha. The other eight plots were not fertilized and were used as controls. We made measurements in two control plots (2 and 3) and two fertilized plots (plots 1 and 4). We did not determine their spatial proximity to each other and because we have background data from many previous studies (Curran et al. 1990; Cooper and Gholt 1991; Gholt et al. 1991).

**Gas fluxes**

We measured $N_2O$, $CH_4$, and $CO_2$ fluxes at the air-soil interface with static chambers on February 2, May 18, and November 2, 1991. These three samplings dates were intended to be sampling replicates to document differences in gas fluxes between control and fertilized soils. At least 12 h before the start of each gas sampling, we placed four chamber wrenches in each of the four plots for a total of 16 chambers per plot. The position of each chamber was marked so that samples were collected at the same location on all three sampling dates. During each gas sampling, two paired plots (one control and one fertilized) were sampled simultaneously at 06:00-06:30, 10:00-10:30, 14:00-14:30, and 18:00-18:30. The second pair was sampled immediately after the first. It took 1.5 hours to sample all four plots. Samples were collected, stored in pressurized 20-ml nylon syringes and analyzed within 1 week of collection at The Ecosystems Center in Woods Hole, Mass. Details of our sampling and analytical techniques can be found in Brandt et al. (1990), Bowden et al. (1990, 1991), and Castro et al. (1993).

**Soil parameters**

During each of the 30-min sampling periods, we measured soil temperatures (0–2.5 cm and 2.5–5.0 cm) at two chambers in each plot with Omega (Stamford, Conn.) dial thermometers. Once on each of the three samplings dates (at approximately 12:00), we collected soil cores from each plot to measure soil moisture, $NH_4$-N and $NO_3$-N concentrations. In February, two soil cores were taken next to two chambers in each plot. In May and November, one core was taken next to all four chambers in each plot. All cores were separated into 0–2 and 2.5 cm increments. Soil moisture was determined gravimetrically; 10-g sub-

---

**Microbial activity**

Immediately after the February gas sampling, we composited two soil cores from each of the four plots (two control and two fertilized), kept the 0–2 and 2.5–5 cm increments separate. This totalled in a total of eight samples. All soil was sieved through a 4-mm window screen. Three 1-g replicate subsamples were run for each of three treatments. The treatments were: (i) $CO_2$ addition to unabated soil; (ii) $CO_2$ and N-Serve (2-chloro-6-(trichloromethyl)pyridine) addition to saturated soils and (iii) $CH_4$ addition to unabated soil. Saturation was required to distribute the N-Serve. These three treatments were necessary to determine the relative activity of the bacteria populations responsible for $CH_4$ oxidation using the technique of Jones et al. (1984).

Methane can be oxidized in soils by methanotrophs and nitifying bacteria. The relative activities of these bacteria in surface soils (0.2–2 and 2.5 cm) from our control and fertilized plots were determined from the ratio of $CH_4$ oxidation to N-Serve sensitive $CO$ oxidation. The N-Serve sensitive $CO$ oxidation is equal to $CH_4$ oxidation by methanotrophs and nitifying bacteria and is calculated as the difference between the $CO$ oxidation rate (treatment 1) and $CO_2$ oxidation rate with N-Serve treatment 2. Since methanotrophs and nitifying bacteria have about the same ability to oxidize $CH_4$, dividing the $CH_4$ oxidation rate by the N-Serve sensitive $CO$ oxidation normalizes the $CH_4$ oxidation rates for the abundance of these bacteria. This is necessary because methanotrophs have a greater (two to four orders of magnitude) ability to oxidize $CH_4$ than nitifying bacteria (Jones et al. 1984). The ratio of $CH_4$ oxidation to N-Serve sensitive $CO$ oxidation was $<0.01$, then nitifying bacteria were primarily responsible for $CH_4$ oxidation in these soils. Higher ratios (≥0.03) suggest that methanotrophs were the dominant group of active $CH_4$ oxidizing bacteria. The 0.01–0.03 ratio was derived from studies conducted with pure culture cultures and environmental samples (R. Jones, personal communication).

**Results**

**Trace gas fluxes**

On each sampling date, the daily average $CO_2$ emissions from the control and fertilized soils were not significantly different (Table 1; Duncan’s multiple range test, $p = 0.05$).

The control and fertilized soils were sources of $N_2O$ (Table 1). On each sampling date, daily averaged $N_2O$ emissions from the fertilized soils were significantly higher (6–600 times) than $N_2O$ emissions from the control soils (Duncan’s multiple range test, $p = 0.05$).

The control and fertilized soils were sinks for atmospheric $CH_4$. On each sampling date, $CH_4$ uptake by the fertilized soils was significantly lower (5–10 times) than $CH_4$ uptake by the control soils (Duncan’s multiple range test, $p = 0.05$).

**Soil parameters**

On each sampling date, there were no significant differences in the daily averaged soil temperatures (0–2.5 and 2.5–5.0 cm) in the control and fertilized soils (t-test, $p = 0.05$).

In general, the average soil moisture in the fertilized and control soils were not significantly different (t-test, $p = 0.05$). However, soil moisture in only the surface soil (0–2 cm) were significantly different in November. At this time, the average soil moisture in the fertilized soil ($65\%$, g H2O/g
dry soil) was ca. two times higher than soil moisture in the control soil.

There were significant (t-test, p = 0.05) treatment differences in the KC1 extractable NH4+, N and NO3-N concentrations. Ammonium-N concentrations were significantly higher (3-10 times) in the fertilized surface soils (0-2 cm) in May and November and deeper soils (2-5 cm) in February and May (Table 1). In addition, only the fertilized soils had detectable NO3-N concentrations (Table 1).

**Microbial activity**

There were two major differences in the relative activities of the microbial populations that oxidized CH4 in the control and fertilized soils (Table 2). First, CH4 oxidation in the control soils was dominated by methanotrophs (i.e., CH4/CO > 0.01); their activity was greatest in the deeper soils (2-5 cm). Second, CH4 oxidation was dominated by nitrifying bacteria in the upper surface (0-2 cm) of the fertilized soil (i.e., CH4/CO < 0.01) while methanotrophs dominated CH4 oxidation in the deeper soils (2-5 cm) of the fertilized plots (Table 2).

**Discussion**

The effect of N fertilization on the growth of pine plantations in the southeastern United States has been well documented, but there is little information about the effects of this silvicultural practice on trace gas fluxes from soils in this ecosystem. In this study, however, we examined the effects of urea-N fertilization on the exchange of N2O, CH4 and CO2 between the atmosphere and soils of a mature slash pine plantation in Florida.

Results from our study suggest that urea N fertilization did not affect CO2 emissions from soils in this plantation during the 4th year of fertilization (Table 1). This result is consistent with results from measurements of CO2 fluxes made in these same plots with the soda lime static chamber technique (W.P. Cropper and H.L. Ghilz, unpublished data) and in situ measurements of fine root respiration (Cropper and Ghilz 1991).

On all three samplings dates, the fertilized soils had significantly higher (8-600 times) daily averaged N2O emissions than control soils (Table 1). This result is consistent with results from many fertilization studies (Duxbury and McCaughney 1988; Hutchinson and Mosier 1979; Keller et al. 1988; McKenney et al. 1980). At our study site, soil NO3-N concentrations were detected in only the fertilized soils (Table 1). Nitrate is a precursor for denitrification and during the conversion of urea to NO3-N, nitrification had to occur. Thus, the increased N2O emissions from the fertilized soils may have been caused by both nitrification and denitrification.

The fertilized soils had significantly lower (5-20 times) CH4 uptake than the control soils (Table 1). This result is consistent with results from N fertilization field experiments conducted in forests (Massachusetts) and grasslands (Colorado) and laboratory experiments conducted with agricultural (Louisiana) and subarctic soils (Quebec) (Mosier et al. 1991; Steudler et al. 1989; Nesbitt and Breitenbeck 1992; Adamsen and King 1993). Collectively, these results suggest that N-induced inhibition of CH4 uptake is a widespread phenomenon and should be considered in global estimates and model forecasts of CH4 budgets.

The mechanism responsible for the effect of N fertilization...
on CH$_4$ uptake by soils is not well known. Currently, there are two hypotheses to explain this effect. First, high inorganic N concentrations in the fertilized soil may lower microbial oxidation of CH$_4$ (Steadler et al. 1989; Melillo et al. 1989). This is supported by laboratory experiments that show that high concentrations of both NO$_3^-$ and NH$_4^+$ inhibit CH$_4$ oxidation by pure cultures of methanotrophs and nitrifying bacteria (Ferretti et al., 1975; Hyman and Wood 1983; Jones and Morita 1983). Results from a laboratory study conducted with fresh soil samples suggest that NH$_4^+$, and not NO$_3^-$, appears to be an irreversible inhibitor of CH$_4$ oxidation and the inhibitory effect persists after oxidation of NH$_4^+$ (Nesbitt and Breitenbeck 1992). Although our data suggest that CH$_4$ uptake was lowest in the soils with the highest NH$_4^+$ and NO$_3^-$ concentrations, our data set was not large enough to establish a strong statistical relationship. Second, results from field studies in grasslands suggest that soil N cycling, rather than inorganic soil N concentrations, influences CH$_4$ uptake by grassland soils (Mosier et al. 1991). Since we did not measure soil N turnover at our study sites, we do not have the appropriate data to examine the effect of N turnover on CH$_4$ uptake.

Results from our measurements of the relative activities of the bacteria responsible for CH$_4$ oxidation suggest that nitrifying bacteria dominated CH$_4$ oxidation in the surface (0–2 cm) of the fertilized soils and methanotrophs dominated CH$_4$ oxidation in the control soils (Table 2). The fertilized soils also had significantly lower CH$_4$ uptake than the control soils (Table 1). This pattern is consistent with laboratory measurements of CH$_4$ oxidation by these bacteria. Pure cultures of nitrifying bacteria had between two to four orders of magnitude lower CH$_4$ oxidation rates than pure cultures of methanotrophs (Jones et al. 1984). Thus, the shift in the relative activities of the microbial populations that oxidize atmospheric CH$_4$ towards bacteria less effective at oxidizing CH$_4$ in response to the N fertilization may have been responsible for the lower CH$_4$ uptake by the fertilized soil. In summary, 4 years of N fertilization of soils in this slash pine plantation did not affect the CO$_2$ emissions, but increased N$_2$O emissions into the atmosphere and lowered the uptake of atmospheric CH$_4$. Alterations in the CH$_4$ fluxes may have resulted from N-induced changes in the relative activities of the soil bacteria responsible for CH$_4$ oxidation. These results imply that the soil N status of this ecosystem affects the atmospheric fluxes of N$_2$O and CH$_4$ and that alterations in the soil N status resulting from N fertilization is likely to affect the exchange of N$_2$O and CH$_4$ between the atmosphere and soils in this ecosystem.

**Acknowledgements**

We thank Jon Chapman and Michelle Millersky for field assistance and the Jefferson-Smarft Container Corporation of America for use of their slash pine plantation. This work was supported by funds from the United States Environmental Protection Agency ( Cooperative agreement CR817734-010, Athens Environmental Laboratory) and National Science Foundation grant BRS-8919647.


Duxbury, J.M., and McCloskey, P.R. 1988. Effects of fertil-
Stone, E.L. 1983. The managed slash pine ecosystem, School of Forest Resources and Conservation, University of Florida, Gainesville.