

## The effect of increased N deposition on nitrous oxide, methane and carbon dioxide fluxes from unmanaged forest and grassland communities in Michigan

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**Abstract.** Atmospheric nitrogen deposition is anticipated to increase over the next decades with possible implications for future forest-atmosphere interactions. Increased soil N<sub>2</sub>O emissions, depressed CH<sub>4</sub> uptake and depressed soil respiration CO<sub>2</sub> loss is considered a likely response to increased N deposition. This study examined fluxes of N<sub>2</sub>O, CH<sub>4</sub> and CO<sub>2</sub> over two growing seasons from soils in unmanaged forest and grassland communities on abandoned agricultural areas in Michigan. All sites were subject to simulated increased N-deposition in the range of 1–3 g N m<sup>-2</sup> annually. Nitrous oxide fluxes and soil N concentrations in coniferous and grassland sites were on the whole unaffected by the increased N-inputs. It is noteworthy though that N<sub>2</sub>O emissions increased three-fold in the coniferous sites in the first growing season in response to the low N treatment, although the response was barely significant ( $p < 0.06$ ). In deciduous forests, we observed increased levels of soil mineral N during the second year of N fertilization, however N<sub>2</sub>O fluxes did not increase. Rates of methane oxidation were similar in all sites with no affect of field N application. Likewise, we did not observe any changes in soil CO<sub>2</sub> efflux in response to N additions. The combination of tillage history and vegetation type was important for the trace gas fluxes, i.e. soil CO<sub>2</sub> efflux was greater in successional grassland sites compared with the forested sites and CH<sub>4</sub> uptake was reduced in post-tillage coniferous- and successional sites compared with the old-growth deciduous site. Our results indicate that short-term increased N availability influenced individual processes linked to trace gas turnover in the soil independently from the ecosystem N status. However, changes in whole system fluxes were not evident and were very likely mediated by competitive N uptake processes.

### Introduction

Concern over increasing atmospheric concentrations of the biogenic greenhouse gases nitrous oxide (N<sub>2</sub>O), methane (CH<sub>4</sub>), and carbon dioxide (CO<sub>2</sub>) has stimulated much work addressing their flux magnitudes from forest ecosystems as well as studies focusing on the environmental and anthropogenic controls on flux intensities. Most often forest ecosystems in the temperate region provide a source of N<sub>2</sub>O to the atmosphere although emission intensities might be small, compared with tropical systems characterized by higher temperatures and greater biological activity (Keller et al. 1983). Despite this

apparent climatic limitation on N<sub>2</sub>O emissions from temperate forests, many studies have shown that N<sub>2</sub>O emissions increase substantially upon artificial nitrogen additions (e.g. Melillo et al. 1989; Brumme and Beese 1992; Matson et al. 1992; Klemetsson et al. 1997; Papen et al. 2001) indicating a key role of nitrogen in the control of N<sub>2</sub>O emission rates. Regions with elevated atmospheric N-deposition due to anthropogenic activity also show increased N<sub>2</sub>O emissions (Butterbach-Bahl et al. 1998, 2002).

Temperate forests are significant sinks for CH<sub>4</sub> (Adamsen and King 1993; MacDonald et al. 1996; Robertson et al. 2000). The main regulator of soil CH<sub>4</sub> oxidation appears to be the diffusion of methane into the soil (Brumme and Borken 1999). There is evidence that the sink strength of CH<sub>4</sub> can be suppressed by additions of nitrogen (Stuedler et al. 1989; Adamsen and King 1993; Wang and Ineson 2003; Bodelier and Laanbroek 2004). However, several other studies in forest systems found little or no response of CH<sub>4</sub> oxidation to N additions (Whalen and Reeburgh 2000; Bradford et al. 2001; Steinkamp et al. 2001). Increased atmospheric N-inputs have also been demonstrated to reduce soil CH<sub>4</sub> oxidation (Butterbach-Bahl et al. 2002).

Soil respiration of CO<sub>2</sub> is the dominant source by which plant assimilated carbon is returned to the atmosphere. Changes in the magnitude of soil CO<sub>2</sub> efflux due to changes in environmental conditions will likely influence the global atmospheric CO<sub>2</sub> budget (Schlesinger and Andrews 2000). Increased N-inputs, for example, have been shown to retard mineralization of forest soil organic matter (Hagedorn et al. 2003) and depress soil respiration CO<sub>2</sub> losses (Mattson 1995; Bowden et al. 2000; Butnor et al. 2003). However, others have observed small increases in soil respiration in response to increased N-inputs (Brumme and Beese 1992).

Enhanced nitrogen deposition is anticipated in the next few decades due to increased human activity (Galloway et al. 1994; Mosier et al. 2002). It is hypothesized that increased N deposition will result in increased N<sub>2</sub>O emissions in combination with reduced soil CH<sub>4</sub> oxidation and reduced soil respiration CO<sub>2</sub> losses.

In past studies of trace gas fluxes from forest and grasslands affected by increased N-inputs (e.g. Stuedler et al. 1989; Brumme and Beese 1992; Matson et al. 1992; Mattson 1995; Bowden et al. 2000) investigators have usually applied N at rates substantially above ambient atmospheric N-deposition (i.e.  $\geq 10 \text{ g N m}^{-2} \text{ year}^{-1}$ ) in order to evaluate potential N-effects. Long-term changes in atmospheric N-deposition, however, will likely constitute only a fraction of present ambient deposition rates, and trace gas responses might be accordingly limited.

In this work we report results from the first two years of a study initiated to examine the link between low level N inputs and N<sub>2</sub>O, CH<sub>4</sub> and CO<sub>2</sub> fluxes from soils of old-growth deciduous forests, planted coniferous forests, and successional grassland communities on abandoned agricultural areas in Michigan.

## Materials and methods

### *Study sites*

The experiments were conducted at the Kellogg Biological Station (KBS) Long Term Ecological Research site located in southwest Michigan (85°24' W, 42°24' N). The study was performed in  $n = 3$  replicate sites of old-growth native deciduous forest stands, 40–60 year old coniferous plantations and successional grasslands on land abandoned from farming more the 30 years ago. Dominant vegetation was *Carya glabra*, *Quercus rubra*, and *Q. alba* (deciduous forest), *Pinus resinosa* and *P. strobes* (coniferous plantations), and *Aster pilosus*, *Solidago* spp., mixed grasses, lichens, and various shrubs (grasslands). All of the sites were located within a 4-km<sup>2</sup> area on similar soils, typic Hapludalfs ranging from loam to sandy loam textures (Table 1). Annual precipitation at KBS averages 890 mm with about half falling as snow. Mean annual temperature is 9.7 °C. The annual atmospheric N-deposition in the region is about 0.6 g N m<sup>-2</sup> (<http://nadp.sws.uiuc.edu>).

### *Field sampling*

Measurements of gas fluxes took place during the growing seasons in 1995 and 1996. Nitrogen application was initiated at the onset of the experiment, and included three treatments, viz. a zero N control (0 N), 1 g N m<sup>-2</sup> year<sup>-1</sup> (1 N)

Table 1. Soil A-horizon characteristics of the different study sites.

Site	Texture	Horizon (cm)	pH	Total N (g kg <sup>-1</sup> soil)	Total C (g kg <sup>-1</sup> soil)	Bulk density (g cm <sup>-3</sup> )
<i>Deciduous forest (DF)</i>		<i>A</i>				
DF1	Loam	11	5.6	2.4	28.9	1.6
DF2	Sandy loam	12	5.9	2.2	26.1	1.6
DF3	Sandy loam	12	4.9	2.2	23.5	1.6
<i>Coniferous forest (CF)</i>		<i>Ap</i>				
CF1	Sandy loam	22	4.1	1.0	15.7	1.6
CF2	Sandy loam	22	4.6	0.7	10.0	1.6
CF3	Loam	19	5.2	1.0	9.1	1.6
<i>Successional community (SC)</i>		<i>Ap</i>				
SC1	Sandy loam	24	5.4	1.0	7.5	1.5
SC2	Loam	20	4.5	1.8	21.6	1.6
SC3	Sandy loam	24	5.0	0.6	5.8	1.5

Numbers are means of duplicate analysis on composite soil samples.

and  $3 \text{ g N m}^{-2} \text{ year}^{-1}$  (3 N). The sites were thus subject to a 2.5- to 6-fold increase in annual N input. The N was added as an  $\text{NH}_4\text{NO}_3$  solution ( $4.43 \text{ g N l}^{-1}$ ) distributed in 4–5 doses during the growing season, beginning in April and ending in November. The 1 N treatment was applied to one  $10 \times 10 \text{ m}$  plot in each site, which received  $0.045 \text{ l m}^{-2}$  of the  $\text{NH}_4\text{NO}_3$  solution at each application using a backpack sprayer. The 3 N treatment was applied to one  $2 \times 2 \text{ m}$  plot in each site, which received  $0.135 \text{ l m}^{-2}$  of the same solution at each application. The 3 N treatment was not applied to the successional grasslands. The 0 N controls were not treated with any solution. Application dates were 13 April, 17 May, 7 August and 9 November in the first year, and 11 April, 14 June, 19 July, 23 September and 18 November in the second year. In the first year a double dose was given on 13 April. The application events preceded gas sampling by at least 2 days. The amounts of solution applied to the 1 N and 3 N plots comprised 0.3–1 ‰ of the annual precipitation, and had no influence on the moisture conditions in the surface soil layers but remained merely as a mist on the surface of soil and litter. Spraying on the ground vegetation, which was particular abundant in the grassland, was avoided in order to limit foliar uptake of N. Due to the limited amount of solution applied, significant infiltration of the applied N to the top soil layers would be dependent on subsequent rainfall events. A potential loss of the applied N as ammonia ( $\text{NH}_3$ ) was considered negligible due to the acidic soil conditions, which prevents transformation of applied  $\text{NH}_4^+$  to  $\text{NH}_3$  (Vlek and Stumpe 1978).

Soil-atmosphere gas fluxes were measured using two-piece static gas chambers (Ambus and Robertson 1999). Duplicate chamber bases made of  $27 \times 27 \times 10 \text{ cm}$  high aluminum frames were permanently installed 8 cm into the ground in each experimental plot. When gas sampling took place, a  $29 \times 29 \times 14 \text{ cm}$  high white ABS plastic cover was fitted into water filled groove on top of the aluminum base, providing a 12-l gas tight enclosure. A rubber septum in the lid allowed gas sampling using a needle and syringe. The chamber remained sealed for 3 h and four headspace samples were removed at 1 h intervals for analysis of  $\text{CH}_4$ ,  $\text{N}_2\text{O}$  and  $\text{CO}_2$ .

Gas flux measurements took place on a bimonthly to monthly basis during the growing season. Systematic baseline gas flux measurements in the 0 N plots were paralleled by measurements in the 1 N-treated in all sites during the first growing season, and occasional measurements were done in the 3 N plots. In the second growing season, systematic measurements took place also in the 3 N plots in the forested sites, whereas gas sampling in the successional grasslands was discontinued. In order to assess effects of the increased N-inputs, cumulative seasonal gas flux rates were then calculated by linear interpolations between sampling dates and averaged ( $n = 3$ ) for each combination of vegetation type and N-treatment. Data from the 3 N plots in the first growing season were disregarded due to the limited sampling frequency.

Sampling involved a three-step procedure. First, the chamber headspace gas was mixed by vigorously pumping the 30-ml sampling syringe several times.

Second, a 2-ml crimp-sealed vial containing ambient air was flushed with 15 ml of headspace sample using a spare hypodermic needle to vent the vial. Finally, a 5-ml sample was withdrawn from the chamber and injected into the now pressurized vial. Gas samples were analyzed 1–2 days after collection to avoid concentration changes by diffusion through the septa, which could be observed beyond 1 week of storage. Concentrations of  $\text{N}_2\text{O}$  in 0.5 ml samples were determined by gas chromatography using a HP 5890 GC equipped with Porapak Q column (80 °C), electron-capture detector (350 °C) and Ar: $\text{CH}_4$  (90:10) carrier at 30 ml  $\text{min}^{-1}$ . Calibration of the ECD was performed by injecting variable amounts of a gas mixture containing 740 ( $\pm 1\%$ ) nl  $\text{l}^{-1}$  of  $\text{N}_2\text{O}$  in synthetic air (Matheson, Montgomeryville, PA, USA). The corresponding calibration curves were fitted by linear regressions ( $R^2 = 0.99$ ) over the range of sample concentrations encountered, usually in the range from 330 to 900 nl  $\text{N}_2\text{O l}^{-1}$ . For  $\text{CH}_4$  determinations (0.5 ml samples) we used a HP 5890 equipped with Porapak Q column (80 °C), flame ionization detector (125 °C) and He carrier at 30 ml  $\text{min}^{-1}$ . Calibration standard was a 1980 ( $\pm 1\%$ ) nl  $\text{l}^{-1}$  mixture of  $\text{CH}_4$  in synthetic air and calibration procedure was as for  $\text{N}_2\text{O}$ . Methane concentrations in samples typically ranged from 400 to 1800 nl  $\text{CH}_4 \text{l}^{-1}$ . Concentrations of  $\text{CO}_2$  were obtained from 1-ml samples analyzed on an infrared gas analyzer (IRGA; Beckman model 865). The IRGA was calibrated at each sampling event using a dilution series made from a gas mixture of 7.1% vol  $\text{CO}_2$  in synthetic air. Sample concentrations frequently ranged between 400 and 4300  $\mu\text{l CO}_2 \text{l}^{-1}$  with peak maxima of 8200  $\mu\text{l l}^{-1}$ .

Gas fluxes were calculated from the change in gas concentration inside the flux chamber over the three hr enclosure period. The relationship between headspace gas concentrations vs. time appeared significantly linear for all three examined components, i.e.  $R^2$  ( $\pm 1\text{SE}$ ) averaged  $0.96 \pm 0.002$ ,  $0.91 \pm 0.005$  and  $0.99 \pm 0.002$ , respectively for  $\text{CH}_4$ ,  $\text{N}_2\text{O}$  and  $\text{CO}_2$ . Flux rates were thus calculated from the slope of the linear regression lines.

Soil inorganic N and gravimetric moisture were analyzed on a monthly basis as part of the KBS-LTER soil-monitoring program (<http://lter.kbs.msu.edu>). Auger samples ( $n = 5$ ; 0–20 cm depth) were collected in each plant community site at monthly intervals during April to November. Soil samples from the fertilized plots were also collected but replication was reduced ( $n = 2$ ) and the small 3 N-treatment plots were sampled less frequently in order to minimize disturbance. Triplicate sub-samples (10 g fresh weight) from a composite soil sample were extracted in 100 ml of 1 M KCl following sieving (4 mm mesh). Filtrates (Type A/E, 1  $\mu\text{m}$  pore size, glass fiber filter) were refrigerated until analyzed for  $\text{NO}_3^-$  and  $\text{NH}_4^+$  performed on an Alpkem 3550 segmented flow analyzer (OI Analytical, College Station, TX, USA). Soil moisture was obtained gravimetrically after oven drying to constant weight (65 °C; 48 h) and is reported as dry weight basis.

Soil pH, total N and total C reported in Table 1 were obtained from the KBS-LTER annual soil sampling in April 1995. pH was measured in soil:water (1:2 w:vol) suspensions. Total N and C were detected from oven dried, finely

ground soil samples using an automated elemental analyzer (Carlo Erba NA1500).

Information on air temperature and precipitation was obtained from the KBS LTER Site Weather Station located in conjunction with the experimental sites.

#### *Laboratory experiment*

The response in trace gas fluxes to N application in the two forest types was further studied in a laboratory based dose-response experiment. For this purpose intact soil columns in 7.5 cm inner diameter PVC-cylinders were sampled to a depth of 12.5 cm in one deciduous forest site and in one coniferous forest site. Sampling took place in October of 1996. Within the same day as sampling, the cylinders containing the soil columns were sealed at the bottom by wrapping with pieces of polyethylene plastic and placed on trays filled with one cm of water and incubated at 20 °C. Variable amounts of  $\text{NH}_4\text{NO}_3$  in a 40-ml dose, equivalent to 0, 0.2, 1, 3 and 10  $\text{g N m}^{-2}$  were then added to four replicate columns. Twenty-four hours after N additions the headspace around each cylinder was sealed by a 15 cm inner diameter by 17 cm high metal can placed open end down to achieve a gas tight seal. The cans were pierced by stoppers for gas sampling, which was done by removing a 5-ml headspace sample at 4-h intervals over a 12-h period. Gas sample storage, gas analysis and rate calculations were performed as previously described except for  $\text{CH}_4$  uptake kinetics, which was considered 1st-order in the laboratory experiment due to the relatively long time lapse.

An additional set of three columns was collected at each site to investigate the vertical profile distribution of gas production and consumption. The columns were sliced into five segments at 2.5 cm increments, which subsequently were composited and sieved (2 mm). Triplicate 20-g portions of the composite samples were weighed into 160-ml serum bottles, sealed and incubated over 6 h to measure the evolution of  $\text{CH}_4$  and  $\text{CO}_2$  by sampling the headspace at two hr intervals. Another set of triplicate 10-g subsamples was weighed into 120-ml serum bottles and amended with 10 ml of a  $\text{KNO}_3$ -solution (100  $\text{mg N l}^{-1}$ ). The bottles were sealed and flushed with  $\text{N}_2$  to remove oxygen from the headspace and incubated for three hrs. The potential evolution of  $\text{N}_2\text{O}$  was then measured by sampling the headspace every hour.

#### *Statistical analysis*

Data comparisons were made using the *t*-test, Anova and Multicomp packages of S-PLUS® (Insightful Corporation, Seattle, USA). In order to meet assumptions of equal variances all gas fluxes were log-transformed prior to statistical analysis.

## Results

### *Climate and soil conditions*

Average air temperatures on a monthly basis ranged between 0.7 and 25.1 °C during the 1995 growing season and between 1.6 and 23.1 °C during the 1996 growing season. For the same periods, the cumulative precipitation reached 837 mm and 662 mm, respectively. The greater precipitation during 1995 was also reflected in wetter soil conditions during this period compared with 1996 (Figure 1). As a whole, however, the seasonal difference was only significant for the deciduous forest control plots (Table 2). Soil total N in the old-growth deciduous forest exceeded soil total N in the conifer plantations and successional communities by 2.5 times and 2 times, respectively (Table 1). Total C in the deciduous sites exceeded total C in both conifer and successional sites by a factor of 2.3. The average levels of soil inorganic N were quite uniform across all sites and N-treatments with a range between 4.0 and 8.3 mg N kg dry soil<sup>-1</sup>. A significant effect of N-application was observed in the deciduous forests in 1996, where soil inorganic N increased from 5.7 to 8.3 mg N kg dry soil<sup>-1</sup>. Soil inorganic N-levels appeared lowest in the successional sites, but they were not significantly different from the two forest systems (Table 2).

### *Gas fluxes in the field*

Nitrous oxide emission was observed in all the sites with a peak activity during the first part of the growing season. The N<sub>2</sub>O emissions did generally not exceed 30 μg N m<sup>-2</sup> h<sup>-1</sup> in untreated plots (Figure 2). Minor peaks in N<sub>2</sub>O emission activity were observed on 11 August and 21 August. Both of these

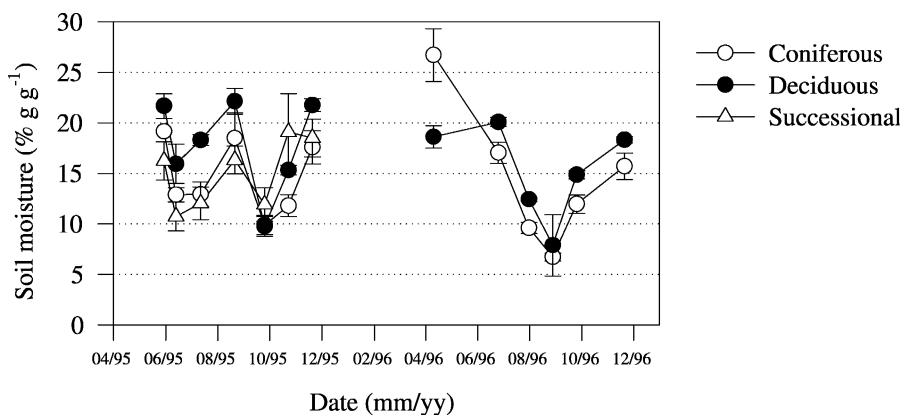


Figure 1. Gravimetric soil moisture content for individual vegetation types determined over two successive growing seasons. Data are means of  $n = 3$  replicates ( $\pm 1$  SE).

Table 2. Seasonal means of total inorganic N and gravimetric soil moisture for the experimental sites and treatments.

Site-treatment	Inorganic N (mg kg <sup>-1</sup> )		Soil moisture (% g g <sup>-1</sup> )	
	1995	1996	1995	1996
<i>Coniferous forest</i>				
0 N	5.9 ± 0.6	7.5 ± 0.5	14.7 ± 1.0	14.6 ± 0.9
1 N	5.7 ± 1.0	7.2 ± 0.8	13.9 ± 1.6	11.7 ± 1.0
3 N	nd	8.3 ± 0.5	nd	10.7 ± 1.4
<i>Deciduous forest</i>				
0 N	6.0 ± 0.8	<b>5.7 ± 0.4</b>	17.8 ± 0.3 <sup>a</sup>	<b>15.4 ± 0.8<sup>b</sup></b>
1 N	7.2 ± 1.0	<b>6.1 ± 0.2</b>	17.1 ± 2.0	<b>15.0 ± 0.5</b>
3 N	nd	<b>8.3 ± 0.3</b>	nd	<b>12.5 ± 0.5</b>
<i>Successional community</i>				
0 N	4.0 ± 0.9	nd	15.0 ± 1.4	nd
1 N	4.3 ± 0.7	nd	14.1 ± 1.5	nd
3 N	nd	nd	nd	nd

The numbers are arithmetic means of  $n = 5-7$  observations ( $\pm 1$ SE).

Different superscript letters denote significant seasonal differences for each combination of vegetation type and N-treatment ( $t$ -test;  $p < 0.05$ ).

Numbers in bold denote significant differences between N-treated plots for each combination of vegetation type and season (Anova;  $p < 0.05$ ).

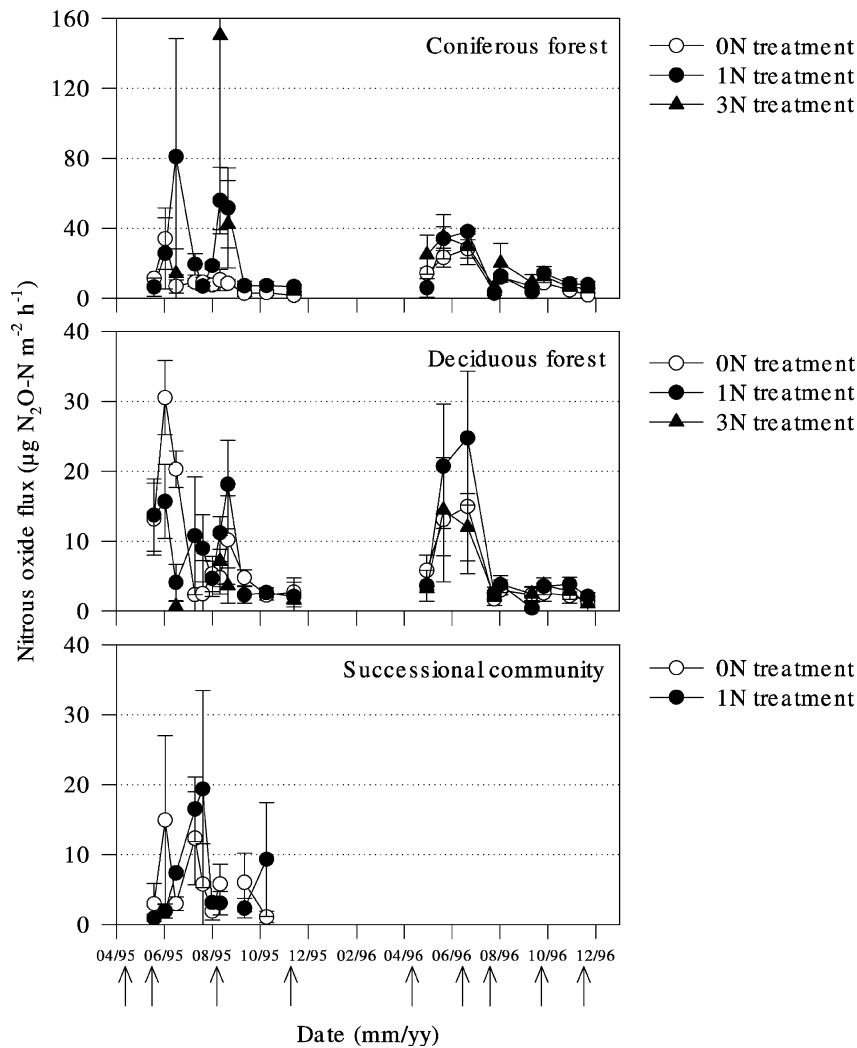
nd = not determined.

sampling events were characterized by substantial amounts of rainfall during the preceding weeks, where 42 mm and 85 mm of precipitation, respectively, was observed. These precipitation events also led to a marked mid-season increase in soil moisture content (Figure 1). In contrast, the peaks in N<sub>2</sub>O activity observed on 2 June and 15 June could not be linked to specific climatic events. Nitrous oxide emissions appeared to increase occasionally in response to the N treatments, e.g. in June and August 1995 in the conifers, in July and August 1995 and June 1996 in the deciduous sites, and in July and October 1995 in the successional sites. However, the daily flux observations were never significantly different between the treatments, apart from the observations on 16 June in the deciduous sites when N<sub>2</sub>O emissions in the control plots significantly exceeded N<sub>2</sub>O emissions in the N treated plots (Figure 2, middle).

The baseline gas flux measurements in 0 N plots indicated a net CH<sub>4</sub> uptake in all sites with peak activity during the mid season (Figure 3). Methane uptake rates peaked at 40–50  $\mu\text{g C m}^{-2} \text{h}^{-1}$  in the old-growth deciduous forest whereas CH<sub>4</sub> uptake in the conifer plantations and successional grasslands rarely exceeded 30  $\mu\text{g C m}^{-2} \text{h}^{-1}$  (Figure 3). In all sites, there was a drop in CH<sub>4</sub> uptake activity on 11 and 21 August 1995. A response to the N treatments was not obvious in any of the vegetation types except in the deciduous forest on 11 September 1996 when CH<sub>4</sub> uptake in the 1 N plots was less than in control and 3 N plots (Figure 3, middle).

Soil CO<sub>2</sub> efflux revealed a distinct seasonal pattern with peak activity in July and August for all sites in the 1995 season (Figure 4). In the 1996 season,





*Figure 2.* The seasonal dynamics of  $\text{N}_2\text{O}$  emissions observed in three different vegetation types in combination with three N treatments. Fluxes were obtained for one (successional grassland) or two successive (coniferous and deciduous forests) growing seasons. Data are arithmetic means ( $\pm 1$  SE) of flux determinations in  $n = 3$  replicate vegetation types. Arrows below the abscissa indicate N fertilization events. Notice different scales on ordinate axis.

maximum activity was observed on 21 June followed by an early-season drop in  $\text{CO}_2$  activity. This sudden drop in  $\text{CO}_2$  activity, which was observed 25 July, coincided with an excessive decrease in soil moisture during July and August when the moisture content in the top 0–20 cm soil reached values as low as  $6\% \text{ g g}^{-1}$  (Figure 1). There was no apparent response in soil  $\text{CO}_2$  efflux to the N treatments.

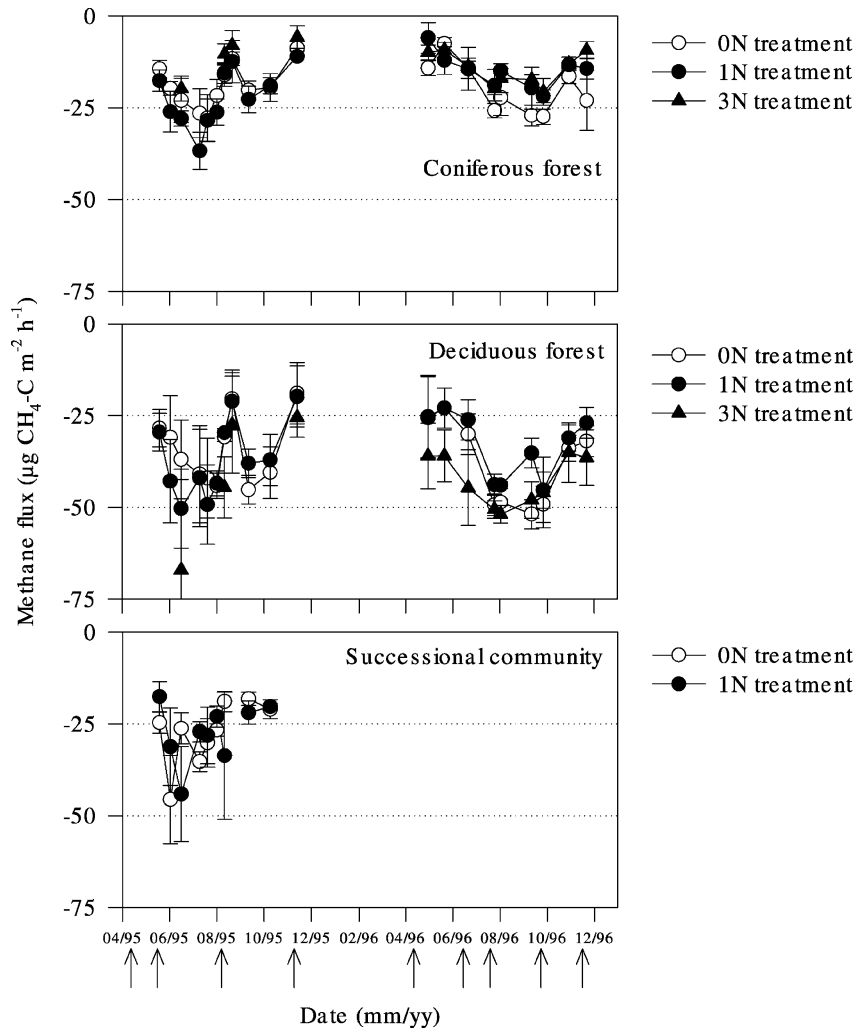


Figure 3. The seasonal dynamics of  $\text{CH}_4$  uptake. For further explanation, see legend for Figure 2.

Cumulative flux data from the 1995 season (Table 3) generally shows that the 1 N treatment had no effect on gas fluxes in any combination of vegetation type and gas species. However, the data suggested ( $p < 0.06$ ) that  $\text{N}_2\text{O}$  emissions from the coniferous forests increased by  $>60 \text{ mg N}_2\text{O-N m}^{-2}$  in response to the 1 N treatment, corresponding to a three-fold increase. A comparison of cumulative gas fluxes from the 0 N plots among the three vegetation types indicated that vegetation type had no effect on cumulative  $\text{N}_2\text{O}$  fluxes in the 1995 season (Table 3). Methane uptake was significantly ( $p < 0.05$ ) greater in the deciduous forest than in the conifer systems whereas the successional sites

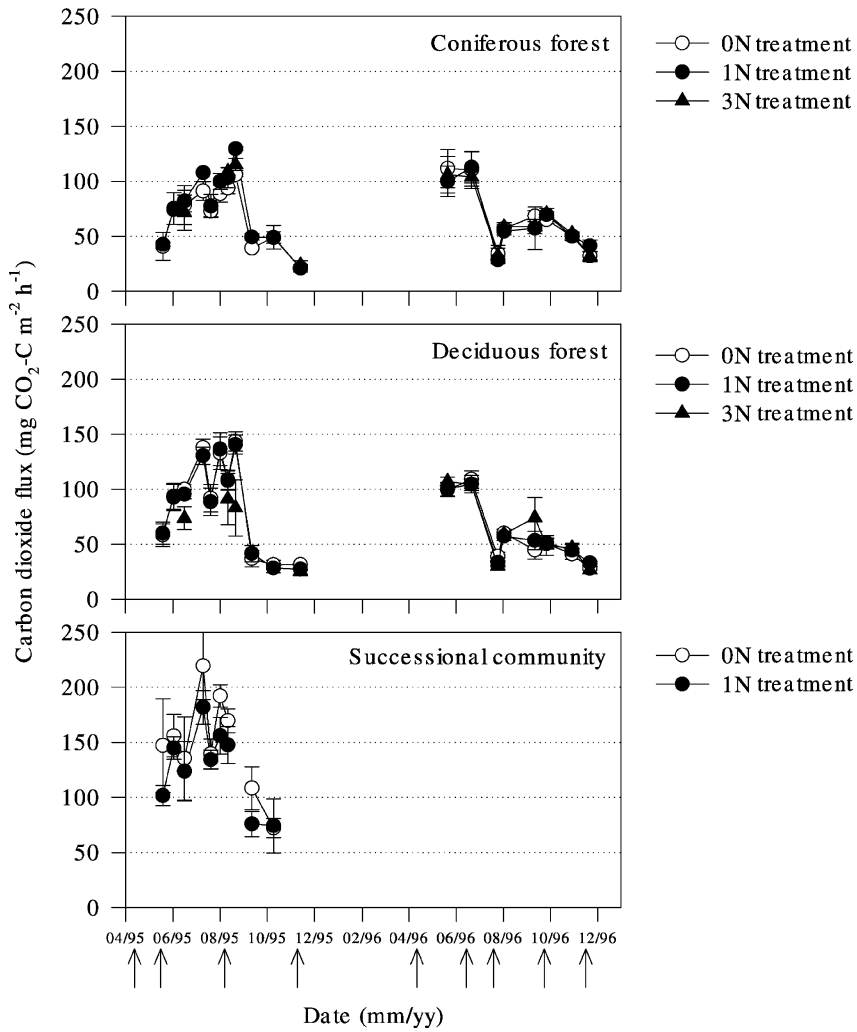


Figure 4. The seasonal dynamics of soil CO<sub>2</sub> effluxes. For further explanation, see legend for Figure 2.

showed an intermediate CH<sub>4</sub> uptake not different from any of the forest systems (Table 3). Soil CO<sub>2</sub> effluxes were significantly ( $p < 0.05$ ) affected by vegetation type in the 1995 season with maximum fluxes observed in the successional grasslands and minimum fluxes in the coniferous sites.

Cumulative fluxes for the 1996 season also indicated a lack of response to the N treatments for any combination of vegetation type and gas species (Table 3). In contrast to the 1995 season, however, the N<sub>2</sub>O emission from the coniferous sites exceeded that from the deciduous sites almost two-fold ( $p < 0.05$ ). For CH<sub>4</sub> uptake, the data confirmed results from the 1995 season, namely a

Table 3. Cumulative fluxes of N<sub>2</sub>O, CH<sub>4</sub> and CO<sub>2</sub> for each combination of vegetation type and N-application rate ( $n = 3 \pm 1$  SE).

Site	N <sub>2</sub> O emission (mg N m <sup>-2</sup> )		CH <sub>4</sub> uptake (mg C m <sup>-2</sup> )		CO <sub>2</sub> emission (g C m <sup>-2</sup> )	
	1995	1996	1995	1996	1995	1996
<i>Coniferous Forest</i>						
0 N	33 ± 10	62 ± 5 <sup>a</sup>	78 ± 11 <sup>a</sup>	96 ± 9 <sup>a</sup>	262 ± 18 <sup>a</sup>	331 ± 29
1 N	100 ± 39	79 ± 9	91 ± 10	77 ± 14	293 ± 14	319 ± 29
3 N	nd	85 ± 33	nd	73 ± 6	nd	321 ± 19
<i>Deciduous Forest</i>						
0 N	34 ± 3	28 ± 7 <sup>b</sup>	145 ± 20 <sup>b</sup>	191 ± 11 <sup>b</sup>	326 ± 46 <sup>a</sup>	295 ± 26
1 N	29 ± 7	42 ± 15	152 ± 25	166 ± 18	318 ± 36	298 ± 12
3 N	nd	28 ± 10	nd	213 ± 33	nd	313 ± 14
<i>Successional community</i>						
0 N	21 ± 4	nd	90 ± 12 <sup>ab</sup>	nd	501 ± 36 <sup>b</sup>	nd
1 N	23 ± 1	nd	94 ± 18	nd	425 ± 25	nd

The numbers have been interpolated from  $n = 9$ – $11$  observations during each growing season. Different superscript letters indicate significantly different gas fluxes between the vegetation types for each combination of gas type and growing season ( $p < 0.05$ ).  
nd = not determined.

significant higher ( $p < 0.01$ ) CH<sub>4</sub> uptake in the old-growth deciduous forest than in the coniferous forest. Belowground respiration CO<sub>2</sub> losses were not different between the two forest types in 1996 (Table 3). However, the trend in the data suggests that respiration was greater in the conifer plantations than in the deciduous forest in 1996, which is opposite to the trend in the data from the 1995 season. Inter-seasonal variability was not observed for any combination of gas species and forest type.

#### *Gas flux N-response in laboratory experiment*

Soil samples from both coniferous and deciduous forest types showed significant ( $p < 0.01$ ) increases in N<sub>2</sub>O emissions in response to incremental N additions (Figure 5a). For both forest soils we observed an exponential increase in N<sub>2</sub>O flux, which correlated significantly to the incremental N treatments. For both soils it was also observed, that N<sub>2</sub>O emissions did not respond to the 0.2 g N m<sup>-2</sup> treatment corresponding to a single dose at the lowest field application rate. Emissions at the 10 g N m<sup>-2</sup> treatment increased about 10-fold. With respect to CH<sub>4</sub> uptake, the two forest systems responded quite differently to the N treatment. In columns from the deciduous forest, CH<sub>4</sub> uptake was significantly ( $p < 0.001$ ) reduced at the successive N applications, and even at the lowest application CH<sub>4</sub> uptake was reduced by 33% (Figure 5b). Methane uptake in soil columns from the coniferous site was generally more than 10-fold lower than CH<sub>4</sub> uptake in soil from the deciduous site, and unresponsive to the N-treatments. Concerning belowground respiration none of the forest systems responded to the N treatments as revealed by an

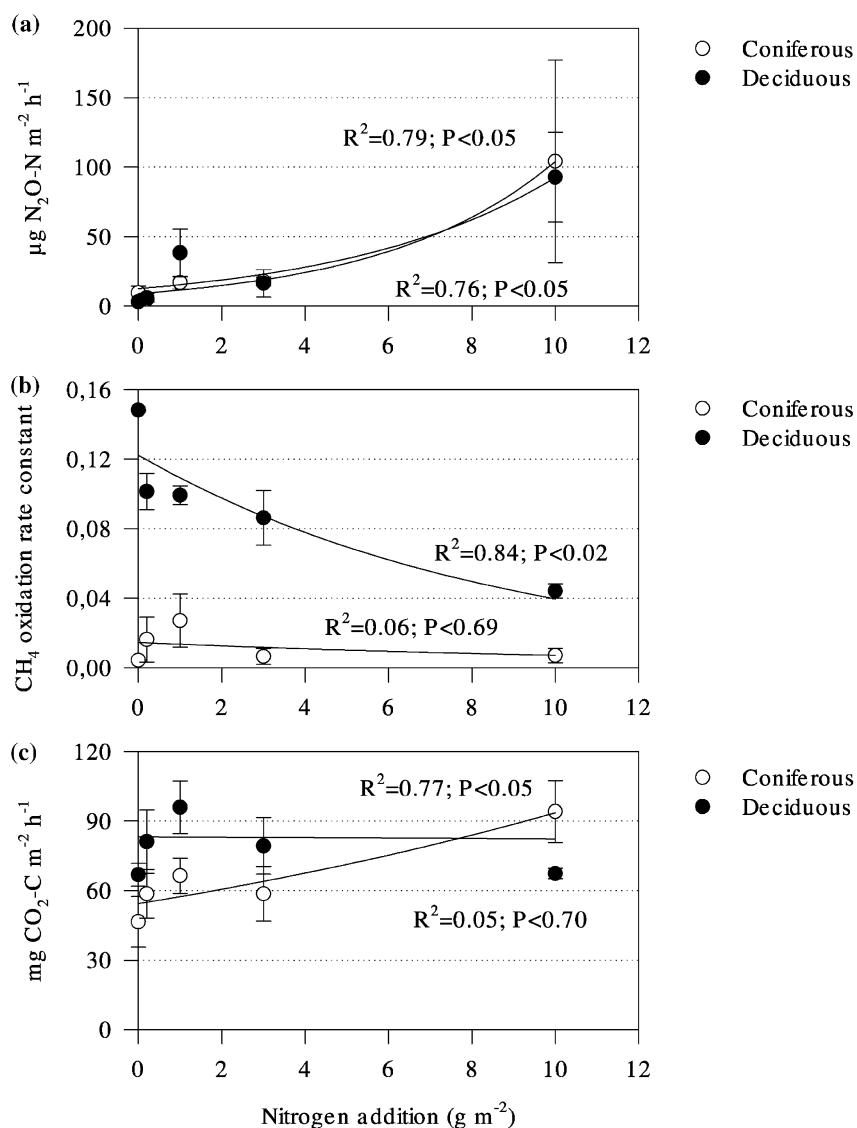


Figure 5. Fluxes of  $\text{N}_2\text{O}$  (a),  $\text{CH}_4$  (b) and  $\text{CO}_2$  (c) from intact soil columns incubated in the laboratory and exposed to varying amounts of N. Each observation represents the mean of  $n = 4$  columns ( $\pm 1$  SE) collected in one deciduous and one coniferous site. Lines indicate exponential fits to the arithmetical means for each vegetation type. Statistical parameters were derived from linear regressions on log-transformed N addition data in order to meet assumptions of normalized data.

analysis of variance considering N input as main factor. However, average  $\text{CO}_2$  emissions in the conifer soils were positively correlated ( $p < 0.05$ ) to the incremental N-treatments (Figure 5c).

Figure 6 pictures the vertical distribution of  $\text{N}_2\text{O}$ ,  $\text{CH}_4$  and  $\text{CO}_2$  activity. In order to compare between the two forest types, the rates have been normalized to peak activity for each combination of gas species and sampling site. With respect to both  $\text{N}_2\text{O}$  and  $\text{CO}_2$  production (Figure 6a, c) we observed a steady vertical decline in activity by depth. For  $\text{CH}_4$  the two sites showed very different patterns with an almost uniform profile activity in the deciduous forest contrasted by a steep increase in activity by depth in the coniferous site (Figure 6b).

## Discussion

### *Impact of increased N deposition*

The current cumulated emissions of  $\text{N}_2\text{O}$  from the forested sites (21–62  $\text{mg N m}^{-2}$ ) are very similar to those reported in a separate study in the same sites (Robertson et al. 2000). Other works on  $\text{N}_2\text{O}$  emissions in temperate forest regions of North America and Europe indicate that the current estimates are intermediate compared to reported values ranging from 1 to 514  $\text{mg N m}^{-2}$  (Table 4).

In our study we applied N at relatively small rates to simulate realistic increases in atmospheric inputs. Nitrous oxide effluxes as well as soil N concentrations in the coniferous and successional grassland sites were unaffected by our increased N-inputs. This lack of response suggests that these systems were not saturated with respect to N such that the additional inputs did not exceed the combined plant and microbial demand for growth (Aber et al. 1989). The coniferous sites, however, did show a strong trend towards a three-fold increase in  $\text{N}_2\text{O}$  emissions, but the N response appeared only during the first growing season and was barely significant ( $p < 0.06$ ). In the deciduous forest, we observed increased levels of mineral N in the 1996 season, which may indicate that these old-growth deciduous forests are approaching N saturation. The additional input of N was apparently in excess of the whole system demand, although not paralleled by increased losses of  $\text{N}_2\text{O}$  as hypothesized by Skiba et al. (1999).

In contrast to the field results, our laboratory experiments indicated substantial  $\text{N}_2\text{O}$  responses to N-inputs. This suggests that, not only the N status of the system but also the N distribution in the soil profile is important for  $\text{N}_2\text{O}$  response. In the field, we added the N with very small amounts of solution (0.045–0.135  $\text{l m}^{-2}$ ) in order to not affect ambient soil moisture. In this manner, the N was applied as a mist mainly onto the surface of the forest-/grassland soil and litter. In the column study, the nutrient solution was added at a rate of 9  $\text{l m}^{-2}$ . This facilitated a better distribution of the added N in the soil volume and thereby ensured a faster diffusion of N to the microsites with peak activity of nitrifying or denitrifying microorganisms and with less

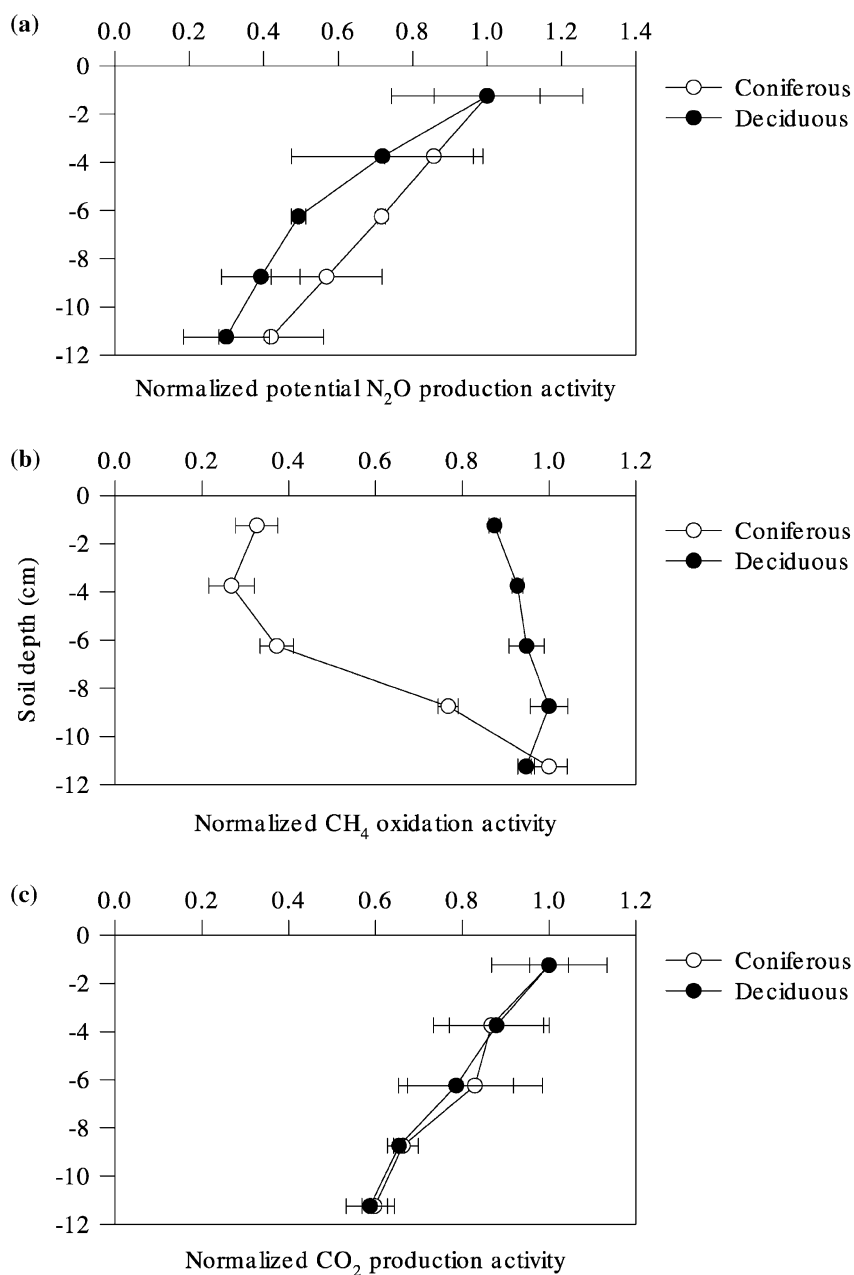


Figure 6. Vertical profiles of  $N_2O$  (a),  $CH_4$  (b) and  $CO_2$  (c) activity in the 0–12 cm top soil in one deciduous and one coniferous site. Each point is the mean of  $n = 3 (\pm 1$  SE) replicate soil samples.

Table 4. Cumulative seasonal emissions of N<sub>2</sub>O reported for different temperate forested sites in North America and Europe.

Study site	Cumulative N <sub>2</sub> O emissions (mg N <sub>2</sub> O-N m <sup>-2</sup> )	Reference
Massachusetts pine- and deciduous forests	1–2	Bowden et al. (1990)
NE United States spruce fir forests	1.5–7.8	Castro et al. (1993)
Pennsylvania deciduous forest	7.5	Bowden et al. (2000)
KBS forests	21–62	Current study
KBS forest	32	Robertson et al. (2000)
German spruce forest	18–83	Butterbach-Bahl et al. (1997)
Wisconsin coniferous and deciduous forests	21–201	Goodroad and Keeney (1984)
German beech forest	45–514	Butterbach-Bahl et al. (1997)
Danish beech forest	80–304	Ambus and Christensen (1995), Ambus et al. (2001)

competition from fungi and other bacteria. In our soil depth experiment, peak N<sub>2</sub>O production was observed in the uppermost 0–2 cm for both deciduous and coniferous soils. Such distribution is presumably controlled by the surface input of litter, suggesting that a few mm of infiltration of an applied N solution would be adequate to ensure exposure to the N<sub>2</sub>O producing microorganisms. From this it follows that a modified field N application approach ensuring a greater infiltration depth might have led to a more marked increase in N<sub>2</sub>O emissions, at least during short time periods after application events. However, the present method was chosen to simulate rainfall driven N input and avoid biased N<sub>2</sub>O emission patterns in response to artificial changes in combined soil moisture and N conditions. It should also be noticed, that the controlled additions of 0.2 g N m<sup>-2</sup> did not induce a response in N<sub>2</sub>O emissions, which confirms the field observations.

As discussed by many authors, N<sub>2</sub>O emissions from forest floors are closely linked with N-cycling, and differences in N<sub>2</sub>O fluxes can be explained in part by differences in atmospheric deposition. Large emissions from German forests (Butterbach-Bahl et al. 1997) are explained as a response to high atmospheric N deposition (2.0–3.5 g m<sup>-2</sup> year<sup>-1</sup>). On the other hand, the low emission rates reported by Castro et al. (1993) and Bowden et al. (1990) were also observed in regions subject to relatively high N-inputs, but these sites were characterized by low, internal net nitrification rates and concomitantly small rates of N<sub>2</sub>O production. Forested ecosystems that are not N saturated (Aber et al. 1989) might show no or little response in N<sub>2</sub>O emissions when exposed to increased inputs over relatively short time periods (2–3 years) as deposited N tends to be immobilized (Skiba et al. 1999). Many forest ecosystems in North



America remain nitrogen limited (Fenn et al. 1998) so longer term changes in N-deposition magnitudes will likely have only modest effect on the trace gas emissions. This also emphasizes the need for long-term studies in order to investigate whole system responses to increased N deposition, at least when N-inputs are low. The lack of response in the current study could possibly change if investigations were continued for a longer time period.

Nitrous oxide is only one gaseous form of N that can be lost in particular from forest soils subject to increased N input. Other investigations have found that emissions of nitric oxide (NO), which is an important catalyst in atmospheric ozone chemistry, is particularly responsive to N additions when losses of NO may exceed losses of N<sub>2</sub>O (Butterbach-Bahl et al. 1997; Skiba et al. 1999; Venterea et al. 2003). These studies also demonstrated that NO losses are higher from coniferous forest soils than from deciduous soils. Studies on forest-atmosphere interactions in response to accelerated N deposition should therefore include also assessments on NO flux rates in order to improve ecosystem N budgets and help predict future impacts on atmospheric chemistry. It can thus be speculated whether NO losses responded in the current experiment, an obvious topic for future investigations in these Michigan ecosystems.

We observed consistent CH<sub>4</sub> uptake in the different sites in agreement with the observations in numerous other studies, including others at KBS (Robertson et al. 2000). Methane uptake rates for temperate deciduous forest, compiled by Bowden et al. (2000), suggest an overall average of 300 mg C m<sup>-2</sup> (range 50–554; *n* = 13) when considering a seven month period. For boreal and temperate forests, Bradford et al. (2001) estimated average uptake rates to fall within 307–455 mg C m<sup>-2</sup> (*n* = 39). Methane uptake rates encountered in this study, ranging from 78 to 191 mg C m<sup>-2</sup>, are thus in the lower range of those reported elsewhere and about 2- to 4-fold lower than a 'global' average. Methane fluxes were not clearly affected by the field N application in any combination of site and season; e.g. in the coniferous sites there was a trend for increasing CH<sub>4</sub> uptake with N additions during the 1995 season, but in the 1996 season this trend was reversed. Although it is well documented that CH<sub>4</sub> uptake can be depressed by increased availability of inorganic N, our results are supported by several other field studies demonstrating no response in CH<sub>4</sub> uptake to N applications at similar or even higher application rates (e.g. Whalen and Reeburgh 2000). As for N<sub>2</sub>O responses it can also be argued that the lack of response to N application is related to the N delivery method, i.e. leaving the added N on the soil surface vs. adding it directly to mineral soil. This is partly confirmed by the observations in the laboratory experiment where CH<sub>4</sub> uptake in soil columns from deciduous forest was strongly inhibited when N was added at a greater infiltration rate. In the coniferous forest, CH<sub>4</sub> uptake activity was characterized by a steep vertical gradient with peak activity below 10 cm depth, as seen in other studies (Crill 1991; Adamsen and King 1993). This vertical distribution means that soil sampling only in the 0–12 cm depth probably is not representative of the total CH<sub>4</sub> uptake in the forest floor. Furthermore, increased N availability in the uppermost soil layers would have

little implication for the total CH<sub>4</sub> oxidation activity, in agreement with our observations from columns from the coniferous forest. A strong CH<sub>4</sub> response to N in such sites would require a vertical infiltration of N to the deeper layers of peak methanotrophic activity. It can also be speculated that a cumulated effect of long-term exposure (several years) to modest N-inputs would affect N<sub>2</sub>O and CH<sub>4</sub> fluxes in these systems, e.g. as observed in prairie systems by Mosier et al. (1991).

The CO<sub>2</sub> fluxes from belowground respiration (range 260–500 g C m<sup>-2</sup>) are within the range of soil CO<sub>2</sub> fluxes observed in other deciduous (Brumme and Beese 1992; Bowden et al. 2000) and coniferous forests (Mattson 1995; Buchmann 2000) in the temperate region, and also indicate a relatively high productivity for the sites in this study (Bowden et al. 2000). Wagai et al. (1998) measured an annual soil CO<sub>2</sub> flux of 719 g C m<sup>-2</sup> from a restored prairie grassland in southern Wisconsin, and Frank et al. (2002) observed a comparable loss of 728 g C m<sup>-2</sup> from prairie grasslands in North Dakota. Frank et al. (2002) estimated the growing season flux in a mixed grass prairie to be 630 g C m<sup>-2</sup>, which is 26% higher than the growing season flux in our grassland community. However, soil CO<sub>2</sub> fluxes are very sensitive to soil temperature fluctuations (Frank et al. 2002), complicating comparisons of studies with different sampling strategies. In our study, sites were most often visited during morning and early afternoon when soil temperatures have not reached maxima, whereas in the study by Frank et al. sampling took place in the afternoon and these authors demonstrated that CO<sub>2</sub> effluxes increased by as much as 30% between 10:00 and 16:00 h.

We observed no evidence for changes in soil respiration in response to the N additions in any combination of site and addition rate. This result is in line with observations by Micks et al. (2004) who found that CO<sub>2</sub> effluxes in the Harvard Forest long term fertilization experiment remained unchanged upon five years of chronic N additions, and these authors suggest that abiotic mechanisms might play a role in forest N retention. In contrast, several other investigations have demonstrated reduced soil respiration in response to increased N availability (Mattson 1995; Bowden et al. 2000; Butnor et al. 2003) due perhaps to a combination of reduced root respiration when N becomes more available and reduced microbial demand for recalcitrant forms of N-containing organic matter (Bowden et al. 2000). This hypothesis appears equivocal as other studies have demonstrated increasing soil respiration in response to N fertilization (Brumme and Beese 1992; Craine et al. 2001). These contradictory results of course emphasize that fertilization responses are likely related to the fertility and N-status of the specific sites making generalization difficult. Moreover, in all the studies cited above the annual N-application rates were at least three-fold higher (range 10–90 g N m<sup>-2</sup>) than in our study, which would make potential N-responses more likely. Apart from the deciduous forest in the 1996 season, we did not observe increases in soil N concentrations, which further indicates that our low N-application rates had limited impact on the N-status and associated processes.

*Impact of land use and vegetation on the trace gas emissions*

Soil management and type of vegetation exerts a strong control on the plant- and microbial mediated soil organic C- and N-cycling, the soil physio-chemical characteristics, soil stability and development. From this perspective it is thus not surprising that numerous works have reported that the exchange of trace gases between soil-plant systems and the atmosphere is to a large degree controlled by land use and vegetation characteristics.

The higher levels of N and C in the old-growth deciduous forests than in the conifer plantations and successional grassland sites implies that possible losses of organic matter due to the previous tillage have not been recovered either by the conifer plantations or by the successional grassland sites. A similar observation was made by Ross et al. (1999) in New Zealand, who found that conversion of deciduous forest to pasture led to a dramatic decrease in soil organic matter whereas a re-introduction of pine trees onto the pastures had little influence on soil organic C 19 years post re-introduction. Nevertheless, we observed significantly greater soil respiration CO<sub>2</sub> losses from the successional grassland than from the two forested sites, which had comparable CO<sub>2</sub> emissions. Differences between grassland and forest soil respiration have been observed in other studies, with 20% higher respiration in grass compared to forests (Raich and Tufekcioglu 2000 and references therein), probably because grasses have more photosynthate available for belowground allocation with virtually no allocation of C to wood production.

In our study, soil respiration did not differ between the two forest types. This is contrasted by the work of Euskirchen et al. (2003) who found that pine barrens in Wisconsin had lower soil respiration compared with mature hardwoods, and by the meta-analysis by Raich and Tufekcioglu (2000) showing that coniferous forests have 10% lower soil respiration than deciduous forest. We found a similar trend in the 1995 season, but this trend was reversed during the 1996 season. It has been suggested that the soil respiration differences between coniferous and deciduous forest types might be due to dissimilarities in litter quality, litter input and root respiration rates (Raich and Tufekcioglu 2000). However, the results from our study indicate that interseasonal variability may perhaps exists suggesting that climatic controls are also important.

Dissimilarities in litter shape have been reported by Brumme and Borken (1999) to affect trace gas emissions in different forest types. These authors reported that CH<sub>4</sub> oxidation was impeded due to reduced gas diffusion through the litter layer under broad-leafed beech compared with spruce forest. In contrast, we found CH<sub>4</sub> uptake to be consistently stronger in the deciduous forest compared with the conifers and successional grassland indicating that other controlling factors were important in our study sites. Our observations are in agreement with other works showing a post-tillage repression of CH<sub>4</sub> uptake even decades after soil management has been reversed to undisturbed grassland (Ojima et al. 1993) or forest (Hudgens and Yavitt 1997). Cultivation depresses CH<sub>4</sub> oxidation (Mosier et al. 1997; Hütsch 1998) and presumably the

pre-tillage  $\text{CH}_4$  oxidation activity in the conifer and successional grassland soils has not fully recovered during the 3–6 decades post-abandonment.

We found no persistent differences in  $\text{N}_2\text{O}$  emissions among the three different vegetation types for the 1995 growing season suggesting that tillage has had no residual impact on  $\text{N}_2\text{O}$  fluxes in combination with the dominant vegetation. This contrasts with the observations by Teepe et al. (2000) who reported a higher  $\text{N}_2\text{O}$  flux from nine-year German fallow grassland compared with a 32-year old oak forest. A 50-year fallow grassland, on the other hand, had less  $\text{N}_2\text{O}$  evolution compared to 30-year beech- and spruce forests (Ambus and Christensen 1995). In the 1996 season, we found the  $\text{N}_2\text{O}$  emission to be higher from the coniferous sites than from the deciduous, very similar to the results achieved by Goodroad and Keeney (1984) comparing a black oak forest with replanted (40-years) white pine in Wisconsin. Certainly it can be argued that past land use practices might be masked by present vegetation types as differences in plant species and associated differentiated inputs of litter quantity and quality, root development and influences on soil microbial activity and physio-chemical soil conditions altogether are important drivers for trace gas turnover in the soil. However, results in the literature are not conclusive. Butterbach-Bahl et al. (1997), for example, found  $\text{N}_2\text{O}$  emissions from German deciduous beech forests to exceed emission from coniferous spruce sites whereas Bowden et al. (1990) observed the opposite relationship comparing Massachusetts red pine and mixed hardwood forests, and Ambus and Christensen (1995) observed similar  $\text{N}_2\text{O}$  fluxes in Danish beech and spruce forests.

## Conclusions

We measured  $\text{N}_2\text{O}$ ,  $\text{CH}_4$  and  $\text{CO}_2$  soil fluxes in old-growth forests, conifer plantations and successional grassland communities in Michigan over two successive growing seasons under ambient conditions as well as under conditions with modest N-inputs. Results show that trace gas fluxes as well as soil mineral N levels in the conifer plantations and successional communities on the whole were unresponsive to increased N-inputs over this time-period, although the data suggested a strong trend towards a three-fold  $\text{N}_2\text{O}$  emission increase in the conifer plantations during one growing season. In the old-growth forest sites increased levels of soil mineral N were observed under increased N-input conditions, although this was not paralleled by increased  $\text{N}_2\text{O}$  emissions. When N was added at greater rates under controlled laboratory conditions we observed significant increases in  $\text{N}_2\text{O}$  fluxes as well as reduced  $\text{CH}_4$  uptake. Overall, the results indicate that increased N availability influenced individual processes linked to trace gas turnover in the soil independently from the ecosystem N status. Whole system fluxes were very likely mediated by competitive N-uptake processes, e.g. plant and microbial growth, and are thus not expected to change until the system approaches N-saturation, which likely will require

long-term N inputs. The results also emphasize the appropriateness of using realistic levels rather than saturating levels of N-inputs in attempts to simulate increased N-deposition.

Important to the trace gas fluxes was also the combination of post-tillage and vegetation type suggesting increased soil respiration in successional grassland sites, reduced CH<sub>4</sub> uptake in post-tillage sites and increased N<sub>2</sub>O emissions from coniferous forest types.

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### References

- Aber J.D., Nadelhoffer K.J., Steudler P. and Melillo J.M. 1989. Nitrogen saturation in northern forest ecosystems. *BioScience* 39: 378–386.
- Adamsen A.P.S. and King G.M. 1993. Methane consumption in temperate and subarctic forest soils: rates, vertical zonation, and responses to water and nitrogen. *Appl. Environ. Microb.* 59: 485–490.
- Ambus P. and Christensen S. 1995. Spatial and seasonal nitrous oxide and methane fluxes in Danish forest-, grassland-, and agroecosystems. *J. Environ. Qual.* 24: 993–1001.
- Ambus P., Jensen J.M., Priemé A., Pilegaard K. and Kjeller A. 2001. Assessment of CH<sub>4</sub> and N<sub>2</sub>O fluxes in a Danish beech (*Fagus sylvatica*) forest and an adjacent N-fertilised barley (*Hordeum vulgare*) field: effects of sewage sludge amendments. *Nutr. Cycl. Agroecosys.* 60: 15–21.
- Ambus P. and Robertson G.P. 1999. Fluxes of CH<sub>4</sub> and N<sub>2</sub>O in aspen stands grown under ambient and twice-ambient CO<sub>2</sub>. *Plant Soil* 209: 1–8.
- Bodelier P.L.E. and Laanbroek H.J. 2004. Nitrogen as a regulatory factor of methane oxidation in soils and sediments. *FEMS Microbiol. Ecol.* 47: 265–277.
- Bowden R.D., Rullo G., Stevens G.R. and Steudler P.A. 2000. Soil fluxes of carbon dioxide, nitrous oxide, and methane at a productive temperate deciduous forest. *J. Environ. Qual.* 29: 268–276.
- Bowden R.D., Steudler P.A. and Melillo J.M. 1990. Annual nitrous oxide fluxes from temperate forest in the Northeastern United States. *J. Geophys. Res.* 95: 13997–14005.
- Bradford M.A., Wookey P.A., Ineson P. and Lappin-Scott H.M. 2001. Controlling factors and effects of chronic nitrogen and sulphur deposition on methane oxidation in a temperate forest soil. *Soil Biol. Biochem.* 33: 93–102.
- Brumme R. and Beese F. 1992. Effects of liming and nitrogen fertilization on emissions of CO<sub>2</sub> and N<sub>2</sub>O from a temperate forest. *J. Geophys. Res.* 97: 12851–12858.
- Brumme R. and Borken W. 1999. Site variation in methane oxidation as affected by atmospheric deposition and type of temperate forest ecosystem. *Global Biogeochem. Cy.* 13: 493–501.
- Buchmann N. 2000. Biotic and abiotic factors controlling soil respiration rates in *Picea abies* stands. *Soil Biol. Biochem.* 32: 1625–1635.

- Butnor J.R., Johnsen K.H., Oren R. and Katul G.G. 2003. Reduction of forest floor respiration by fertilization on both carbon dioxide-enriched and reference 17-year-old loblolly pine stands. *Glob. Change Biol.* 9: 849–861.
- Butterbach-Bahl K., Breuer L., Gasche R., Willibald G. and Papen H. 2002. Exchange of trace gases between soils and the atmosphere in Scots pine forest ecosystems of the northeastern German lowlands 1. Fluxes of N<sub>2</sub>O, NO/NO<sub>2</sub> and CH<sub>4</sub> at forest sites with different N-deposition. *Forest Ecol. Manag.* 167: 123–134.
- Butterbach-Bahl K., Gasche R., Breuer L. and Papen H. 1997. Fluxes of NO and N<sub>2</sub>O from temperate forest soils: impact of forest type, N deposition and of liming on the NO and N<sub>2</sub>O emissions. *Nutr. Cycl. Agroecosys.* 48: 79–90.
- Butterbach-Bahl K., Gasche R., Huber C.H., Kreutzer K. and Papen H. 1998. Impact of N-input by wet deposition on N-trace gas fluxes and CH<sub>4</sub>-oxidation in spruce forest ecosystems of the temperate zone in Europe. *Atmos. Environ.* 32: 559–564.
- Castro M.S., Steudler P.A., Melillo J.M., Aber J.D. and Millham S. 1993. Exchange of N<sub>2</sub>O and CH<sub>4</sub> between the atmosphere and soils in spruce-fir forests in the Northeastern United-States. *Biogeochemistry*, 18: 119–135.
- Craine J.M., Wedin D.A. and Reich P.B. 2001. The response of soil CO<sub>2</sub> flux to changes in atmospheric CO<sub>2</sub>, nitrogen supply and plant diversity. *Glob. Change Biol.* 7: 947–953.
- Crill P.M. 1991. Seasonal patterns of methane uptake and carbon dioxide release by a temperate woodland soil. *Global Biogeochem. Cy.* 5: 319–334.
- Euskirchen E.S., Chen J., Gustafson E.J. and Ma S. 2003. Soil respiration at dominant patch types within a managed northern Wisconsin landscape. *Ecosystems* 6: 595–607.
- Fenn M.E., Poth M.A., Aber J.D., Baron J.S., Bormann B.T., Johnson D.W., Lemly A.D., McNulty S.G., Ryan D.E. and Stottlemeyer R. 1998. Nitrogen excess in North American ecosystems: predisposing factors, ecosystem responses, and management strategies. *Ecol. Appl.* 8: 706–733.
- Frank A.B., Liebig M.A. and Hanson J.D. 2002. Soil carbon dioxide fluxes in northern semiarid grasslands. *Soil Biol. Biochem.* 34: 1235–1241.
- Galloway J.N., Levy II H. and Kasibhatla P.S. 1994. Year 2020: Consequences of population growth and development on deposition of oxidized nitrogen. *Ambio* 23: 120–123.
- Goodroad L. and Keeney D. 1984. Nitrous oxide emission from forest, marsh and prairie ecosystems. *J. Environ. Qual.* 13: 448–452.
- Hagedorn F., Spinnler D. and Siegwolf R. 2003. Increased N deposition retards mineralization of old soil organic matter. *Soil Biol. Biochem.* 35: 1683–1692.
- Hudgens E. and Yavitt J.B. 1997. Land-use effects on soil methane and carbon dioxide fluxes in forests near Ithaca, New York. *Ecoscience* 4: 214–222.
- Hütsch B.W. 1998. Tillage and land use effects on methane oxidation rates and their vertical profiles in soil. *Biol. Fertil. Soils* 27: 284–292.
- Keller M., Goreau T., Wofsy S., Kaplan W. and McElroy M. 1983. Production of nitrous oxide and consumption of methane by forest soils. *Geophys. Res. Lett.* 10: 1156–1159.
- Klemetsson L., Klemetsson Å.K., Moldan F. and Weslien P. 1997. Nitrous oxide emission from Swedish forest soil in relation to liming and increased N-deposition. *Biol. Fertil. Soils* 25: 290–295.
- MacDonald J.A., Skiba U., Sheppard L.J., Hargreaves K.J., Smith K.A. and Fowler D.A. 1996. Soil environmental variables affecting the flux of methane from a range of forest, moorland and agricultural soils. *Biogeochemistry* 34: 113–132.
- Matson P., Gower S., Volkman C., Billow C. and Grier C. 1992. Soil nitrogen cycling and nitrous oxide flux in a Rocky Mountain douglas fir forest: effects of fertilization, irrigation and carbon addition. *Biogeochemistry* 18: 101–117.
- Mattson K.G. 1995. CO<sub>2</sub> efflux from coniferous forest soils: comparison of measurement methods and effects of added nitrogen. In: Lal R., Kimble J., Levine E. and Stewart B.A. (eds), *Soils and Global Change*. CRC Lewis Publishers, Boca Raton, Florida, pp. 329–341.
- Melillo J.M., Steudler P.A., Aber J.D. and Bowden R.D. 1989. Atmospheric deposition and nutrient cycling. In: Andreae M.O. and Schimel D.S. (eds), *Exchange of Trace Gases between Terrestrial Ecosystems and the Atmosphere*. John Wiley & Sons. pp. 263–280.

- Mosier A.R., Bleken M.A., Chaiwanakupt P., Ellis E.C., Freney J.R., Howarth R.B., Matson P.A., Minami K., Naylor R., Weeks K.N. and Zhu Z.-L. 2002. Policy implications of human-accelerated nitrogen cycling. *Biogeochemistry* 57/58: 477–516.
- Micks P., Aber J.D., Boone R.D. and Davidson E.A. 2004. Short-term soil respiration and nitrogen immobilization response to nitrogen applications in control and nitrogen-enriched temperate forests. *Forest Ecol. Manag.* 196: 57–70.
- Mosier A.R., Delgado J.A., Cochran V.L., Valentine D.W. and Parton W.J. 1997. Impact of agriculture on soil consumption of atmospheric CH<sub>4</sub> and a comparison of CH<sub>4</sub> and N<sub>2</sub>O flux in subarctic, temperate and tropical grasslands. *Nutr. Cycl. Agroecosys.* 49: 71–83.
- Mosier A., Schimel D., Valentine D., Bronson K. and Parton W. 1991. Methane and nitrous oxide fluxes in native, fertilized and cultivated grasslands. *Nature* 350: 330–332.
- Ojima D.S., Valentine D.W., Mosier A.R., Parton W.J. and Schimel D.S. 1993. Effect of land use change on methane oxidation in temperate forest and grassland soils. *Chemosphere* 26: 675–685.
- Papen H., Daum M., Steinkamp R. and Butterbach-Bahl K. 2001. N<sub>2</sub>O and CH<sub>4</sub>-fluxes from soils of a N-limited and N-fertilized spruce forest ecosystem of the temperate zone. *J. Appl. Bot. Angew. Bot.* 75: 159–163.
- Raich J.W. and Tufekcioglu A. 2000. Vegetation and soil respiration: correlations and controls. *Biogeochemistry* 48: 71–90.
- Robertson G.P., Paul E.A. and Harwood R.R. 2000. Greenhouse gases in intensive agriculture: Contributions of individual gases to the radiative forcing of the atmosphere. *Science* 289: 1922–1925.
- Ross D.J., Tate K.R., Scott N.A. and Feltham C.W. 1999. Land-use change: effects on soil carbon, nitrogen and phosphorus pools and fluxes in three adjacent ecosystems. *Soil Biol. Biochem.* 31: 803–813.
- Schlesinger W.H. and Andrews J.A. 2000. Soil respiration and the global carbon cycle. *Biogeochemistry* 48: 7–20.
- Skiba U., Sheppard L.J., Pitcairn C.E.R., van Dijk S. and Rossall M.J. 1999. The effect of N deposition on nitrous oxide and nitric oxide emissions from temperate forest soils. *Water Air Soil Poll.* 116: 89–98.
- Steinkamp R., Butterbach-Bahl K. and Papen H. 2001. Methane oxidation by soils of an N limited and N fertilized spruce forest in the Black Forest, Germany. *Soil Biol. Biochem.* 33: 145–153.
- Stuedler P.A., Bowden R.D., Melillo J.M. and Aber J.D. 1989. Influence of nitrogen fertilization on methane uptake in temperate forest soils. *Nature* 341: 314–316.
- Teepe R., Brumme R. and Beese F. 2000. Nitrous oxide emissions from frozen soils under agricultural, fallow and forest land. *Soil Biol. Biochem.* 32: 1807–1810.
- Venterea R.T., Groffman P.M., Verchot L.V., Magill A.H., Aber J.D. and Stuedler P.A. 2003. Nitrogen oxide gas emissions from temperate forest soils receiving long-term nitrogen inputs. *Glob. Change Biol.* 9: 346–357.
- Vlek P.L.G. and Stumpe J.M. 1978. Effects of solution chemistry and environmental conditions on ammonia volatilization losses from aqueous systems. *Soil Sci. Soc. Am. J.* 42: 416–421.
- Wagai R., Brye K.R., Gower S.T., Norman J.M. and Bundy L.G. 1998. Land use and environmental factors influencing soil surface CO<sub>2</sub> flux and microbial biomass in natural and managed ecosystems in southern Wisconsin. *Soil Biol. Biochem.* 30: 1501–1509.
- Wang Z.P. and Ineson P. 2003. Methane oxidation in a temperate coniferous forest soil: effects of inorganic N. *Soil Biol. Biochem.* 35: 427–433.
- Whalen S.C. and Reeburgh W.S. 2000. Effect of nitrogen fertilization on atmospheric methane oxidation in boreal forest soils. *Chemosphere – Global Change Sci.* 2: 151–155.