N deposition and elevated CO\textsubscript{2} on methane emissions: Differential responses of indirect effects compared to direct effects through litter chemistry feedbacks

V. A. Pancotto,\textsuperscript{1} P. M. van Bodegom,\textsuperscript{2} J. van Hal,\textsuperscript{2} R. S. P. van Logtestijn,\textsuperscript{2} P. Blokker,\textsuperscript{2} S. Toet,\textsuperscript{2,3} and R. Aerts\textsuperscript{2}

Received 21 July 2009; revised 20 October 2009; accepted 29 October 2009; published 2 April 2010.

[1] Increases in atmospheric CO\textsubscript{2} concentration and N deposition are expected to affect methane (CH\textsubscript{4}) production in soils and emission to the atmosphere, directly through increased plant litter production and indirectly through changes in substrate quality. We examined how CH\textsubscript{4} emission responded to changes in litter quality under increased N and CO\textsubscript{2}, beyond differences in CH\textsubscript{4} resulting from changes in litter production. We used senesced leaves from \textsuperscript{13}C-labeled plants of Molinia caerulea grown at elevated and ambient CO\textsubscript{2} and affected by N fertilization to carry out two experiments: a laboratory litter incubation and a pot experiment. N fertilization increased N and decreased C concentrations in litter whereas elevated CO\textsubscript{2} decreased litter quality as reflected in litter C and N concentrations and in the composition of lignin and saturated fatty acids within the litter. In contrast to our expectations, CH\textsubscript{4} production in the laboratory incubation decreased when using litter from N-fertilized plants as substrate, whereas litter from elevated CO\textsubscript{2} had no effect, compared to controls without N and at ambient CO\textsubscript{2}. Owing to high within-treatment variability in CH\textsubscript{4} emissions, none of the treatment effects were reflected in the pot experiment. C mineralization rates were not affected by any of the treatments. The decrease in CH\textsubscript{4} emissions due to indirect effects of N availability through litter quality changes (described here for the first time) contrast direct effects of N fertilization on CH\textsubscript{4} production. The complex interaction of direct effects with indirect effects of increased N on litter quality may potentially result in a net decrease in CH\textsubscript{4} emissions from wetlands in the long term.


1. Introduction

[2] Methane (CH\textsubscript{4}) is the second most important trace gas after CO\textsubscript{2}. CH\textsubscript{4} concentrations have more than doubled since preindustrial times (1750) and have risen by 1% per year during the last century. Although the rate of increase has slowed to nearly zero during the last decade [Solomon et al., 2007], the most recent measurements show renewed growth from the end 2006 [Rigby et al., 2008].

[3] Atmospheric concentrations of CH\textsubscript{4} are partially determined by soil carbon cycling, given that CH\textsubscript{4} like CO\textsubscript{2} is an end product of soil organic matter decomposition and carbon (C) mineralization [Tsutsuki and Ponnampерум, 1987]. The principal factors controlling methane produc-

\textsuperscript{1}CADIC-CONICET, Ushuaia, Argentina.
\textsuperscript{2}Institute of Ecological Science, Department of Systems Ecology, VU University Amsterdam, Amsterdam, Netherlands.
\textsuperscript{3}Now at Environment Department, University of York, York, UK.

Copyright 2010 by the American Geophysical Union.
0148-0227/10/2009JG001099

1 of 10
lation of plant and litter biomass production [Berendse et al., 2001; Heijmans et al., 2001; Reich et al., 2001]. Such increases in productivity are likely to increase CH₄ emission rates [Chidthaissong and Watanabe, 1997; Saarnio and Silvola, 1999]. Also, CH₄ oxidation (mediated by methane-oxidizing bacteria) serves as an important control on CH₄ emission to the atmosphere and is inhibited at high NH₄ availability [Bodelier et al., 2000].

5] Indirect effects result from changes during plant growth causing differences in the biochemical composition of plants [Norby et al., 2001; Tolvanen and Henry, 2001], which subsequently affect their decomposition rate in the soil and therefore CH₄ production. Increased CO₂ typically changes litter chemistry through an increased production of secondary carbon compounds [Poorter and Navas, 2003]. Although increased N deposition leads to a smaller tissue C/N ratios, subsequent decomposition rates may increase [Aerts and Chapin, 2000; Aerts et al., 2006], decrease [Magill and Aber, 1998; Gorissen and Cotrufo, 2000], or remain unaffected [Hobbie and Vitousek, 2000] depending on litter quality [Knorr et al., 2005]. Parallel to N deposition, an acceleration in N mineralization due to global warming is expected to further increase N availability [Mack et al., 2004]. To our knowledge there has been little research quantifying the effects of changes in litter quality on CH₄ emissions, even though such effects may potentially have global significance.

6] The aim of this paper is to quantify the rate of methane production from litter of plants grown under the influence of increased atmospheric CO₂ and N availability. We expected elevated atmospheric CO₂ and increased N deposition to increase litter production, but that litter quality and hence methane production would be affected differently by these abiotic factors. We hypothesized that elevated CO₂ would lead to more secondary carbon compounds such as lignin and saturated fatty acids (lowering methane production rates), while increased N deposition would increase litter decomposability (raising methane production rates).

7] We performed two complementary experiments: (1) a laboratory litter incubation experiment, where we studied litter mass loss, carbon mineralization and the contribution of CO₂ and CH₄ to these processes as affected only by changed litter quality under controlled, waterlogged conditions to test these hypotheses; and (2) a greenhouse pot experiment with the same litter used in experiment 1 added to waterlogged soil to validate the patterns in CH₄ emission.

2. Materials and Methods

2.1. Plant Material

8] We used Molinia caerulea plants, a dominant species from wetlands throughout Northern and Western Europe, occurring in peatlands with water-saturated conditions in spring and dropping groundwater levels during summer. Methane emissions are known to occur in these systems [Lloyd et al., 1998]. To obtain 13C labeled litter of M. caerulea, intact PVC mesocosms of 25 cm diameter and 40 cm height were cut from a lowland peatland dominated by M. caerulea and Sphagnum palustre. The intact mesocosms were kept in a greenhouse in a factorial design with and without increased N deposition of 60 kg ha⁻¹ yr⁻¹ (N⁺ and N⁻, respectively), corresponding to twice the ambient deposition in The Netherlands; and with and without elevated atmospheric CO₂ concentrations (of 700 and 400 ppm, C⁺ and C⁻, respectively) in 6 replicates (N = 24). Although the mesocosms were kept in a greenhouse, the air temperature followed the natural annual course with minor differences. Nitrogen was applied as NH₄Cl. Other nutrients were applied in nonlimiting amounts: 0.8g P/m², and 6.4 g K/m². During the third growing season, the mesocosms were spiked four times with 13C-enriched atmospheric CO₂ in all treatments and all the naturally senesced litter of this 13C-M. caerulea was subsequently collected to quantify total litter production rates. Six replicate air-dried and ground litter subsamples were analyzed for total organic carbon (C), total nitrogen (N) concentration and δ¹³C values. In addition, the abundance of major organic compounds was determined to evaluate litter quality (see below). This same litter was used in the laboratory litter-incubation experiment and in the greenhouse pot experiment.

2.2. Litter Incubation

9] Rates of carbon mineralization and methane production were measured by incubating litter in glass jars. Glass jars (80 ml) were filled with 1.0 g of 13C-labeled M. caerulea litter cut into 5 cm long fragments. The litter was covered with 20 ml of water collected from remoistened fresh M. caerulea litter that had been soaked overnight in demi-water and filtered over a 100 μm Whatman filter, as litter-specific inoculum [Strickland et al., 2009] and to create waterlogged conditions.

10] We used 6 replicates of litter per treatment combination. In addition, 6 jars without litter were prepared to control for CO₂ and CH₄ produced by the water alone. After closure, all jars were flushed with N₂ at 1 bar for 50 s to generate anoxic conditions [van Bodegom et al., 2005] and incubated them in the dark at a constant temperature of 20°C, optimal for litter decomposition [Aerts and de Caluwe, 1997]. We collected 100 μl headspace with a syringe at 3–4 days intervals to measure CH₄ and CO₂ concentrations and δ¹³C values. After each measurement we flushed again with N₂ and returned the jars to the 20°C incubator.

2.3. Pot Experiment

11] Soil was collected from wet dune slacks in the western part of the Netherlands, an area known to be very poor in organic matter (soil total C = 0.66%, soil total N = 0.02%). The soil was sieved to minimize the contribution from sources of organic material other than the litter and thus maximize the sensitivity to differences in emissions induced by litter. Thirty pots of 0.011 m² and 20 cm high were filled with this soil and planted with M. caerulea. Each pot contained one plant with 4 to 7 tillers, and 13–35 leaves.
Neither initial number of leaves, nor number of tillers or leaf lengths was significantly different between treatments.

[12] All pots were placed in a greenhouse under controlled photoperiod (14 h light and 10 h dark), light intensity (250 μmol m⁻² s⁻¹) and temperature (17°C). In addition, we controlled the water level of the pots, mimicking flooded conditions with 1–2 cm standing water. The pots were equilibrated for 2 months during which time 50 ml of 5 mM acetate was added on a weekly basis to stimulate methane production and electron acceptor consumption. Previous research under similar conditions has indicated that acetate additions decrease the redox potentials to values suitable for methane production, but do not negatively affect plant performance or general microbial activity [van Bodegom et al., 2008].

[13] The ¹³C-labeled *M. caerulea* litter from each replicate of the original treatment combinations was cut into 1 cm long fragments. Litterbags (5 x 9 cm) with 1 mm² mesh size were filled with 0.35 g of this litter and two of them were randomly inserted just below the soil surface of each pot, after equilibration of the pots. In control pots, we simulated bag placement and subsequent root disturbance. This resulted in 4 treatments (given the 4 litter “types”) and 1 control, each replicated 6 times (N = 30).

[14] For gas sample collection, a ventilated chamber (60 cm high with a surface area of 0.011 m²) with water lock was temporarily installed on top of each pot [Matson and Goldstein, 2000]. Gas samples for CH₄ emission determination were collected by allowing gas exchange for 10 s through a double needle with a 5 ml gas vial that was previously evacuated. 5 ml samples of, which 500 μl was used for CH₄ analysis, were collected at 0, 5, 10, 15 and 20 min after positioning of the chamber. CH₄ production rates were calculated using regression assuming linear gas evolution (and rejecting calculated rates if R² < 0.85). At 20 min, an additional gas sample of 100 ml was collected for δ¹³C analysis of accumulated CH₄. The ventilated chamber was removed immediately after sampling. Samples were collected at t = 0, 7, 14, 21, 28, 35, 42, and 49 days to quantify CH₄ emission dynamics from *M. caerulea* litter. No CO₂ was analyzed in this experiment, given the interference of plant photosynthesis and plant respiration on measured CO₂ signals. After 49 days, *M. caerulea* was harvested and the litter remaining in the litterbags was quantified.

2.4. Chemical Analysis

[15] Initial total organic carbon (C) and total nitrogen (N) concentration of the litter was measured on a Perkin Elmer 2400 series II CHNOS/O analyzer. δ¹³C values of the litter were analyzed on a Carlo Erba EA1110 elemental analyzer coupled to a Finnigan Delta Plus IR-GCMS (isotope ratio–gas chromatograph/mass spectrometer).

[16] The relative abundance of major groups of macromolecular organic compounds, like saturated fatty acids, guaiacyl lignin, syringyl lignin, and p-hydroxyphenyl lignin, was determined through chemolysis of thermally assisted hydrolysis and methylation, as the number of detected ions in the mass spectrometer is linearly related to the amount of material [Blokker et al., 2005]. Organic compounds were chosen to represent the components relevant for decomposition and were classified on the basis of their mass spectra, applying the commercial databases Wiley 6 and NIST98 [Yeloff et al., 2008]. To 200 μg of sample, 5 μl of a 25% TMAH solution in methanol was added. The samples were incubated in a pyrolysis liner (CDS) at 70°C for 2 h and pyrolyzed at 550°C for 5 min in a CDS AS-2500 pyrolysis unit (CDS Analytical Inc.) (260°C interface temperature) coupled to an Agilent 6890 GC equipped with an Agilent 5973 MSD. The GC oven was programmed from 40°C (5 min hold time) to 130°C at 20°C/min and subsequently to 320°C at 6°C/min followed by 10 min isothermal at 320°C. A HP5-MS capillary GC column was used with helium as carrier gas at a constant flow of 1.2 ml/min in a 20:1 split ratio. The mass spectrometer was operated in full-scan mode (m/z 50–800) at 70 eV ionization energy [Blokker et al., 2005].

[17] We determined total CH₄, N₂O and CO₂ concentrations on a Hewlett Packard 5890A gas chromatograph equipped with a 25 m Carboxplot P7 column and a flame ionization detector for CH₄ and a thermal conductivity detector for N₂O and CO₂. The minimum flux detection limit was approximately 0.5 ppm CH₄. The δ¹³C values of CH₄ were determined with a Finnigan PreCon/Gasbench coupled to a Finnigan Delta Plus IR-GCMS. For this purpose, we used an analysis routine that first removes all CO₂, second oxidizes all CH₄ to CO₂ and then measures δ¹³C values at an accuracy of 0.1‰. The volume injected into the GCMS was estimated using the CH₄ concentration detected by GC.

2.5. Calculations

[18] In both experiments, measured CH₄ may have been produced from litter or from other sources of organic matter, such as organic matter dissolved in the water or in the soil of the pot experiment. To isolate the effects of litter on CH₄ production, a ¹³C mass balance was used:

\[
\text{AT}^{13}\text{C}_{\text{measured}}/100 \cdot \text{CH}_4\text{measured} = \text{AT}^{13}\text{C}_{\text{o.m.}}/100 \cdot \text{CH}_4\text{o.m.} + \text{AT}^{13}\text{C}_{\text{litter}}/100 \cdot \text{CH}_4\text{litter}
\]

where CH₄ is CH₄ release, and AT⁺¹³C is the atom percentage of ¹³C over total C measured in released CH₄ (to account for fractionation of ¹³C during conversion of organic C to CH₄). For AT⁺¹³C_{litter}, we considered the value corresponding to the last measurement of ¹³C for CH₄ produced from litter at the laboratory litter incubation, assuming that litter was the only source of CH₄ remaining during the last period of incubation. This value was used both for the litter incubation and pot experiments. CH₄ production and AT⁺¹³C from sources of available organic matter other than litter was obtained from controls without litter.

2.6. Statistical Analysis

2.6.1. Litter Quality and Mass Loss

[19] The effects of N fertilization and of CO₂ concentration during plant growth on initial litter C, initial litter N and total litter mass loss in both experiments were analyzed using a two-way ANOVA. Weights of the duplicate litter bags within each pot were averaged. Effects of control versus treatments were tested with one-way repeated measures ANOVA (rmANOVA) with time as within-subjects factor and a Dunnett post hoc test at each time.
Table 1. Litter Parameters From Original Mesocosms

<table>
<thead>
<tr>
<th></th>
<th>C° N°</th>
<th>C° N°</th>
<th>C° N°</th>
<th>C° N°</th>
<th>N Fertilization</th>
<th>CO₂</th>
<th>N*CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litter production (gm⁻² yr⁻¹)</td>
<td>236.5</td>
<td>202.3</td>
<td>186.6</td>
<td>190.6</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>(17.7)</td>
<td>(24.7)</td>
<td>(17)</td>
<td>(27.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Litter C (%)</td>
<td>44.93</td>
<td>45.64</td>
<td>44.93</td>
<td>45.58</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>(0.11)</td>
<td>(0.09)</td>
<td>(0.09)</td>
<td>(0.10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Litter N (%)</td>
<td>0.54</td>
<td>0.94</td>
<td>0.60</td>
<td>0.96</td>
<td>**</td>
<td>NS</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>(0.003)</td>
<td>(0.005)</td>
<td>(0.004)</td>
<td>(0.003)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/N ratio</td>
<td>83.7</td>
<td>48.3</td>
<td>74.2</td>
<td>47.6</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>(0.39)</td>
<td>(0.31)</td>
<td>(0.58)</td>
<td>(0.15)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>δ¹³C values (%)</td>
<td>315</td>
<td>702</td>
<td>293</td>
<td>716</td>
<td>**</td>
<td>NS</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>(3.45)</td>
<td>(3.45)</td>
<td>(1.31)</td>
<td>(4.50)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mean (±SE, given in parentheses, N = 6) litter production from original mesocosms, initial carbon and nitrogen concentration, C/N ratio, δ¹³C value per dry mass of M. caerulea litter from plants grown under the combination of two treatments: CO₂: 400 and 700 ppm CO₂ (C° and C°, respectively) and N fertilization: with and without increased 60 kg ha⁻¹ yr⁻¹ N (N° and N°, respectively). **P < 0.01.

[20] Patterns in composition of major groups of macromolecular organic compounds in the litter were determined by redundancy analysis (RDA), constrained and explained by the N fertilization and CO₂ treatments (direct gradient analysis). A RDA was chosen, because a detrended correspondence data analysis showed that the gradient length of the first axis was around one [Leps and Smilauer, 2003]. Scaling was focused on interspecies correlations, species data (i.e., organic compounds) were centered and explained by the treatments and were significant (P < 0.05 and P < 0.01, respectively). So, the imposed treatments led to strong changes in the composition of pyrolyzed organic compounds, explaining in total 46% of the variation.

[21] The composition of organic macromolecular compounds in the litter was also strongly affected by N fertilization and CO₂ concentration (Figure 1): The first canonical-RDA axis explained 28% of the total variability in the organic compounds, while an additional 18% was explained by the second RDA axis. Both axes were constrained by the treatments and were significant (P < 0.05 and P < 0.01, respectively). The effects of N fertilization on average led to an increase in guaiacyl-lignin fragments, whereas N fertilization on average led to lower abundance of wax compounds. Results were very similar when a RDA using the individual treatments as dummy environmental factors was performed (data not shown).

3.2. Litter Incubation

[25] Based on the lower C/N ratios (Table 1) and lower abundance of wax compounds (Figure 1), increased decomposability upon N fertilization was expected, while the higher C/N ratios and higher guaiacyl-lignin abundance under elevated CO₂ would indicate lower decomposability. However, no large differences in mass loss from M. caerulea litter were observed. After 48 days, approximately 20% of the original litter had been decomposed. Mass loss from litter under ambient CO₂ was about 2% higher than from litter under elevated CO₂ (F = 5.23, P < 0.05, Figure 2a), but there were no N fertilization effects. Treatment effects on cumulative C mineralization over the same time period were even smaller: Cumulative C mineralization was not affected by any of the treatments (Figure 3a). C mineralization rates were higher at the start of the incubation and decreased with time (F = 130.03, P < 0.07), stabilized to linear accumulation rates from the third week onward (Figure 3a). Cumulative C mineralization from controls was significantly lower than from any treatment during the whole experiment (F = 19.96, P < 0.05; Dunnett Test P <
Mass loss of *M. caerulea* and N on CH$_4$ and C$_4$N$_4$(N production increased with time (F = 166.1, p < 0.05) and fertilization treatment (C$^+$N, Figure 4) throughout the experiment. The cumulative CH$_4$ production from litter contributed less than 25% to the total CH$_4$ emissions and was not affected by treatments (Figure 5b), indicating that soil organic matter served as main source of CH$_4$. The high within-treatment variability was unrelated to plant biomass or number of tillers (which have been used as covariables to decrease within-treatment variability in the past [Denier van der Gon and Neue, 1996]). Cumulative CH$_4$ emissions increased until the third and fourth weeks, for C$^+$ treatments and C$^+$ treatments, respectively (Figures 5a and 5b). Thereafter, CH$_4$ production from litter leveled off for all treatments (Figure 5b, time effect F = 181.4 and F = 14.0, P < 0.01 for total CH$_4$ emission and CH$_4$ emission from litter, respectively).

The temporal dynamics of $\delta^{13}$C-CH$_4$ values were synchronous to the CH$_4$ emissions (Figure 6), but remained much lower than the original $\delta^{13}$C values of the litter. The $\delta^{13}$C values of emitted CH$_4$ were significantly higher for treatments with litter grown under N fertilization, compared to those without N fertilization, reflecting the coincidental initial differences in $\delta^{13}$C litter labeling (F = 12.28, P < 0.05). However, this effect varied marginally with time (F = 0.05 at each point in time). The same was true for cumulative CO$_2$ production (data not shown), which was an order of magnitude higher than CH$_4$ production, and which dominated total C mineralization.

Figure 1. Biplot from a redundancy analysis (RDA) with treatments (as environmental variables, arrows) and organic compounds in the litter (species; saturated fatty acids, asterisks; guaiacyl lignin, diamonds; syringyl lignin, squares; p-hydroxyphenyl lignin, circles) determined through chemical analysis of the litter. Axis 1 (horizontal) explains 28% of the total variance, and axis 2 (vertical) explains 18%. Litter was collected from plants grown at 400 and 700 ppm CO$_2$ (C$^+$ and C$^-$, respectively) and with or without increased 60 kg ha$^{-1}$ yr$^{-1}$ N (N$^+$ and N$^-$, respectively).

Figure 2. Mass loss of *M. caerulea* litter from plants in (a) a laboratory experiment incubating litter for 48 days (N = 24) and (b) a greenhouse pot experiment with mass loss measured over 49 days (N = 24). Treatments are as explained for Figure 1, and each bar represents the mean and standard error of the mean.

3.3. Pot Experiment

[Litter decomposition of *M. caerulea* was not affected by the treatments (Figure 2b). After 45 days, approximately 20% from the original litter had decomposed.](#)

Cumulative CH$_4$ emissions from the pots were highly variable within treatments, and no treatment effect was detected (Figure 5a). CH$_4$ produced from litter contributed less than 25% to the total CH$_4$ emissions and was not affected by treatments (Figure 5b), indicating that soil organic matter served as main source of CH$_4$. The high within-treatment variability was unrelated to plant biomass or number of tillers (which have been used as covariables to decrease within-treatment variability in the past [Denier van der Gon and Neue, 1996]). Cumulative CH$_4$ emissions increased until the third and fourth weeks, for C$^+$ treatments and C$^+$ treatments, respectively (Figures 5a and 5b). Thereafter, CH$_4$ production from litter leveled off for all treatments (Figure 5b, time effect F = 181.4 and F = 14.0, P < 0.01 for total CH$_4$ emission and CH$_4$ emission from litter, respectively).

The temporal dynamics of $\delta^{13}$C-CH$_4$ values were synchronous to the CH$_4$ emissions (Figure 6), but remained much lower than the original $\delta^{13}$C values of the litter. The $\delta^{13}$C values of emitted CH$_4$ were significantly higher for treatments with litter grown under N fertilization, compared to those without N fertilization, reflecting the coincidental initial differences in $\delta^{13}$C litter labeling (F = 12.28, P < 0.05). However, this effect varied marginally with time (F = 0.05 at each point in time). The same was true for cumulative CO$_2$ production (data not shown), which was an order of magnitude higher than CH$_4$ production, and which dominated total C mineralization.

Patterns in cumulative total CH$_4$ production (Figure 3b) and the calculated cumulative CH$_4$ produced from the litter (Figure 3c) were similar, reflecting that CH$_4$ production rates in the controls were constant in time. Only the treatments without N fertilization produced more CH$_4$ than the controls (Dunnett Test P < 0.05 for the fourth week onward). The cumulative CH$_4$ production from litter was lower with N fertilization (F = 43.17, P < 0.01; Figure 3c), but was unaffected by CO$_2$ concentration. However, the N fertilization effect varied with CO$_2$ concentration: Under N$^-$, cumulative CH$_4$ production was higher under ambient CO$_2$ than under elevated CO$_2$, while with N$^+$, CH$_4$ production was higher under elevated CO$_2$ than under ambient CO$_2$. Cumulative CH$_4$ production increased with time (F = 166.1, P < 0.01).

The $\delta^{13}$C-CH$_4$ signature of the treatments was enriched compared to controls (Dunnett test, P < 0.05), that had $\delta^{13}$C values of $-55$ to $-50$‰ (Figure 4) throughout the experiment. The $\delta^{13}$C of CH$_4$ emitted by each treatment approached that of each treatments respective litter $\delta^{13}$C values. $\delta^{13}$C-CH$_4$ attained the litter $\delta^{13}$C values in both treatments without fertilization (N$^-$) after 20 days. Thus, the CH$_4$ produced was mainly derived from *M. caerulea* litter. $\delta^{13}$C-CH$_4$ in the treatment with ambient CO$_2$ and fertilization (C$^-$N$^-$) was similar to that of the initial litter at the start of the incubation, but decreased afterward, while $\delta^{13}$C-CH$_4$ of the elevated CO$_2$ and fertilization treatment (C$^+$N$^+$) always remained lower than the initial value of the corresponding litter. The lower $\delta^{13}$C signal of C$^+$N$^+$ suggests an extra source of CH$_4$ from an alternative organic matter source to litter; this assertion is compatible with the lower decomposition in this treatment.
2.73, P = 0.054, for N fertilization*time interaction) as the differences between N⁺ and N⁻ decreased as the experiment progressed, suggesting that CH₄ production from litter grown in the absence of N fertilization was higher. The CO₂ concentration effect on δ¹³C-CH₄ values also varied with time. At the end of the experiment, elevated CO₂ treatments had higher δ¹³C-CH₄ values than those from the ambient CO₂ treatments (F = 5.18, P < 0.01, for CO₂ concentration*time interaction), whereas initial differences in δ¹³C-CH₄ values were not significant.

4. Discussion and Conclusions

4.1. Litter Quality

[31] The reported decreases in N concentrations under elevated CO₂ and the consequent increases in C/N ratios [Cotrufo and Ineson, 1996; Berntson and Bazzaz, 1998] have been linked to a larger accumulation of carbon-based secondary compounds, like lignins, polyphenols and waxes in plant tissues [Peñuelas et al., 1996; Peñuelas and Estiarte, 1998; Hu et al., 1999]. Indeed, using chemolysis and RDA analysis, we confirmed that elevated CO₂ increased the relative abundance of lignin. Low N availability is associated with increased concentrations of carbon-based secondary compounds [Herms and Mattson, 1992; Hättenschwiler and Vitousek, 2000] and was associated with increased abundance of wax compounds in our study. Even so, the chemolysis analysis, in which the majority of the macromolecular organic compounds were identified, showed that shifts in composition of these compounds were more important than absolute increases in their abundance.

[32] The absolute effects of elevated CO₂ were small compared to those of N fertilization, also reported in several reviews [Hirschel et al., 1997; Norby et al., 2001; Finzi and Schlesinger, 2002]. Overall, the effects of our treatments on litter quality were similar to those typically reported in the literature, although the details of changes were more precisely quantified in our study. This makes our study appropriate to separate the effects of global change factors mediated through litter quality versus litter production on CH₄ production. Unfortunately, to explicitly separate these two factors, a common soil environment had to be used to avoid differences in litter quantity, but as in our study, the use of common soil limits the discrimination of potential changes to the soil microbial community due to elevated CO₂/N deposition (although the effects of potential changes...
in soil microbial community may be marginal) [Allison and Martiny, 2008].

4.2. CH$_4$ Production Responds Differently Compared to Total Carbon Mineralization

[33] Methane production measured in the litter incubation under anoxic conditions followed the expected temporal dynamics, increasing with time following the expected depletion of the pools of alternative electron acceptors from water and M. caerulea litter. At the end of the incubation CH$_4$ predominantly came from litter, as reflected in the $\delta^{13}$C values that approached the $\delta^{13}$C values of the litter itself. The convergence of $\delta^{13}$C values of produced CH$_4$ with those of the labeled litter, in the laboratory litter incubation, provides circumstantial evidence that the litter from different treatments had been labeled uniformly with $^{13}$C.

[34] In the short-term laboratory incubation experiment, the treatments affected CH$_4$ production differently compared to C mineralization, which is an integrative measure of the available carbon substrates for CH$_4$ production. While CH$_4$ production decreased upon N fertilization and was unaffected by CO$_2$ elevation, there was no effect of N fertilization upon C mineralization and litter mass loss, but decreased mass losses of M. caerulea litter at elevated CO$_2$ (the latter coinciding with decreased litter quality [Gorissen et al., 1995; Hirschel et al., 1997]). Thus, differences in the availability of precursors for methane production (like acetate and H$_2$/CO$_2$) did not explain the effects of the global change factors on CH$_4$ production. This also implies that differences in lignin and other secondary carbon compounds, or differences in litter decomposability in general, do not explain the patterns in CH$_4$ production rates in our study.

[35] In our study, decreased CH$_4$ production from fertilized litter was not due to increased denitrification either, which would have been possible due to higher concentrations of litter N, as a secondary compound that acts as alternative electron acceptor [Knowles, 1979]. Using the acetylene inhibitor method, we did not detect N$_2$O in any gas samples, indicating that no detectable denitrification had occurred (data not shown). Low denitrification rates below the detection limit of the system would not have affected CH$_4$ production rates so strongly. If denitrification had occurred during the first days of incubation (acetylene was added after 10 days), then the inhibiting effects of denitrification should have been extinguished afterward. This did not happen, thus we conclude that differences in denitrification do not explain the treatment effects of N fertilization on methanogenesis either.

[36] Alternatively, it has been reported that methanogens may be sensitive to ammonium and gaseous N-containing products [Hendriksen and Ahring, 1991; Klüber and Conrad, 1998; van Bodegom and Scholten, 2001]. Although it is still difficult to determine the mechanism of toxicity and which type of methanogens are affected [Sawayama et al., 2004], this may explain why the pathways of total carbon mineralization and CH$_4$ production were affected differently by N fertilization.

[37] In the pot experiment, effluxes of CH$_4$ were similar to those in the laboratory incubation, but the variability in effluxes within treatments was much higher, obscuring any significant treatment effect. This variability that occurred in soil microbial community may be marginal) [Allison and Martiny, 2008].

Figure 5. CH$_4$ emissions from pots planted with M. caerulea and containing M. caerulea litter in a pot experiment. (a) CH$_4$ emissions measured from pots and (b) calculated CH$_4$ emission from litter. Classification of treatments follows Figure 1.

Figure 6. The $\delta^{13}$C values of CH$_4$ emitted from pots planted with M. caerulea and containing M. caerulea litter in a pot experiment. Classification of treatments follows Figure 1.
under less controlled conditions may reflect that in semi-natural conditions other factors or mechanisms are involved, that we did not control in the pot experiment, and that also affect CH₄ emissions. In our pot experiment, leaf litter was inserted in the water-saturated topsoil layer in order to maximize the contribution of leaf litter to methane, compared to other carbon sources like root materials. This setup should thus maximize the capacity to detect potential indirect effects through litter quality on methane production. Still, treatment effects were insignificant and the δ¹³C labeling of the pot experiment produced much lower values than the laboratory litter incubation, reflecting that litter was a minor source of CH₄ in the pot experiment (compare Figures 5a and 5b [Chidthaisong and Watanabe, 1997; Watanabe et al., 1998; von Fischer and Hedin, 2007]). Note that partial oxidation of methane would increase δ¹³C of emitted methane, overestimating the role of labeled leaf litter to methane emissions and even further decreasing the actual role of this carbon source. The relative contribution of CH₄ from litter was fairly constant, as reflected in the low variation in δ¹³C. The natural variability in the other sources of CH₄ thus probably caused the nonsignificant treatment effects in CH₄ emissions. The relative increase in δ¹³C for N⁻ treatments compared to the N⁺ treatments shows, however, that CH₄ production from unfertilized litter gained importance during the experiment, consistent with the laboratory incubation.

4.3. Potential Consequences of the Differential Response of CH₄ Production

[38] Studies on the effects of climate change on soil carbon respiration [Mack et al., 2004] and litter decomposition [Knorr et al., 2005; Aerts et al., 2006] in peat ecosystems have consistently reported an increase in carbon fluxes under N deposition and elevated CO₂. These effects on litter decomposability have been attributed partly to influences of increased N availability [Mack et al., 2004] as N mineralization rates also rise [Rustad et al., 2001]. Although it is appealing to assume that CH₄ production rates will respond similarly to these indirect effects of global change, as they comprise part of the soil carbon cycle, we found that CH₄ production responds in a different way, as the factors controlling its production seem to be different to those for total carbon respiration.

[39] In contrast to the anticipated increase in concert with other carbon fluxes, and in contrast to potential amplification of responses for methane due to inhibiting effects of N compounds on CH₄ oxidation [Kravchenko, 2002], CH₄ production rates from litter decreased following N fertilization of M. caerulea plants. These decreases were large and ranged from 50% (for litter produced at elevated CO₂ concentrations) to 80% (for litter from ambient CO₂ concentrations). These indirect effects of N availability on CH₄ production, on which hitherto no data were available, also contrast with the combined effects of N fertilization through litter production and litter chemistry on CH₄ production rates. Combined effects for peatlands show only a transitory stimulation of CH₄ efflux [Aerts and Toet, 1997; Saarnio and Silvola, 1999; Nykänen et al., 2002] or no significant response [Dise and Verry, 2001]. This small stimulation is somewhat unexpected in the light of the generally increased litter biomass production upon N fertilization [Berendse et al., 2001; Heijmans et al., 2001; Reich et al., 2001], although there were no significant differences in litter production under the different treatments in our study.

[40] The strong negative effect of N deposition on CH₄ production through litter N availability in this study may explain, however, why the combined effects of N deposition are only modest. This implies that it is important to account for both the direct and indirect effects when predicting CH₄ emissions from wetlands, although presently none of these effects are accounted for in any global CH₄-emission model on wetlands. This may particularly affect predictions for regions with high N deposition [Dentener et al., 2006] and high CH₄ emissions from wetlands [e.g., Shindell et al., 2004], like central Europe and northern China.

[41] Overall, our study shows that the indirect effect of N deposition through changes in litter N availability may potentially depress CH₄ production, and that these effects interact with those induced by differences in atmospheric CO₂ concentrations. More research over various conditions is needed to test the generality of these indirect effects in comparison to direct effects of N availability on CH₄ production.

[42] Acknowledgments. V. Pancotto was partially funded by an external fellowship of CONICET. S. Toet was financially supported by USF grant 98.24 of the Vrije Universiteit Amsterdam to R. Aerts. We thank Matt Robson for the suggestions to improve the manuscript.

References


R. Aerts, P. Blokker, P. M. van Bodegom, J. van Hal, and R. S. P. van Logtestijn, Institute of Ecological Science, Department of Systems Ecology, VU University Amsterdam, De Boelelaan 1085, NL-1081 HV Amsterdam, Netherlands.

V. A. Pancotto, CADIC-CONICET, B. Houssay 200, Ushuaia, Tierra del Fuego 9410, Argentina. (pancotto@agro.uba.ar)

S. Toet, Environment Department, University of York, York YO10 5DD, UK.