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Consequences of nitrogen fertilization on soil methane consumption in a productive temperate deciduous forest

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Abstract To investigate the consequences of long-term N additions on soil CH_4 dynamics, we measured in situ CH_4 uptake rates, soil profiles and kinetics parameters during the growing season in a temperate deciduous forest in northwestern Pennsylvania (Allegheny College Bousson Environmental Forest). Measurements were made in control and adjacent plots amended with 100 kg N ha^{-1} $year^{-1}$ for 8 years. We found that the in situ consumption rates were 0.19±0.02 (mean±SE) for the control and 0.12±0.01 mg CH_4–C m^{-2} h^{-1} for the N treatment, indicating that consumption had been reduced by 35% after 8 years of N amendments. Despite the large difference in rates of consumption, there were no differences in the CH_4 concentration profiles between the control and N-amended plots. Laboratory incubations of CH_4 consumption throughout the soil column (organic horizon and mineral soil depths) showed that rates were greatest in the organic horizon of both control and N-amended soils, although consumption was reduced by 42% in the N-amended plot. However, the rate in the organic horizon was only about 50% the rate measured in organic horizons at other temperate forests. The apparent K_m [$K_{m(app)}$] value in the organic horizon of the control plot was fourfold less than the $K_{m(app)}$ value in the organic horizon of another temperate forest, but similar to the $K_{m(app)}$ values in adjacent plots amended with N for a decade. Unlike results for other temperate forests, $K_{m(app)}$ values at Bousson generally did not decrease with soil depth. These results indicate that N cycling strongly controls the CH_4-consuming community, and suggest that alterations of the N cycle due to N deposition or addition may alter rates and the location of CH_4 consumption by soils, even in soils with high N content and cycling rates.

Keywords Methane consumption · Methane kinetics · Trace gas · Nitrogen fertilization

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Introduction

CH_4 is a powerful greenhouse gas that may have significant consequences on future global warming. It has a relative global warming potential 23 times that of CO_2 over a 100-year time horizon, and is estimated to currently contribute 20% to the radiative force driving global climate change (Intergovernmental Panel on Climate Change 1996). This important role has led to numerous studies on the soil biological processes that regulate the uptake and release of CH_4 at the soil–atmosphere interface (Bedard and Knowles 1989; Steudler et al. 1989; Bender and Conrad 1992; Knowles 1993; Conrad 1995, 1996; Schimel and Gulledge 1998; Chan and Parkin 2001).

Temperate forest systems are generally net consumers of CH_4 (Steudler et al. 1989; Castro et al. 1994a; Lessard et al. 1994; Ambus and Christensen 1995; Prieme and Christensen 1997; Brumme and Borken 1999; Bradford et al. 2001; Butterbach-Bahl et al. 2002; Wang and Ineson 2003). Although they play a role in counteracting the accumulation of CH_4 in the atmosphere, human alterations to these systems may reduce CH_4 consumption, leading to an

increase in CH_4 concentration in the atmosphere (Born et al. 1990; Dörr et al. 1993; Ojima et al. 1993).

Changes in soil N fertility have been shown to decrease CH_4 consumption activity of forested and grassland systems (Steudler et al. 1989; Mosier et al. 1991; Castro et al. 1994b). Decreases in CH_4 consuming capacities can be detected shortly after N additions begin and are known to persist for long periods (Steudler et al. 1989; Nesbit and Breitenbeck 1992; Ojima et al. 1993; Schnell and King 1994; Smith et al. 2000).

This study focuses on the consequences of N deposition and long-term N additions on CH_4 dynamics in control and adjacent repeatedly N-amended soils of a temperate deciduous forest stand. Previous work at the beginning of the fertilization experiment showed overall high N turnover and nitrification rates in the control plots as well as a reduced capacity to consume atmospheric CH_4 in the N-amended soils (Bowden et al. 2000). To investigate the longer-term changes in soil CH_4 dynamics, we measured in situ CH_4 consumption, soil CH_4 profiles, N dynamics and enzymatic affinities {apparent K_m[$K_{m(app)}$], apparent V_{max} [$V_{max(app)}$]} of the CH_4 consumers in control and N-amended plots. In addition, we measured CH_4 consumption rates and soil profiles in the control and N-amended deciduous forest stands in the Harvard Forest Long-Term Research Site (Massachusetts) at approximately the same time so we could compare CH_4 dynamics at a high N-cycling site (Bousson) with a site with much less N input and lower soil N-cycling rates.

Materials and methods

Site description

Bousson experimental forest

The high N cycling site is the Bousson Environmental Research Reserve in northwestern Pennsylvania (41°36′N, 80°2′W). The site is a mixed deciduous forest approximately 80 years old dominated by black cherry (*Prunus serotina* Ehrh) and sugar maple (*Acer saccharum* Marsh). These two species make up 60 and 28% of the 434 Mg ha^{-1} aboveground biomass, respectively. The understory is dominated by small maple saplings and ground cover is dominated by maple seedlings, mayapple (*Podophylum* L.) and trout lily (*Erythronium* L.) (Bowden et al. 2000). Litterfall is 2.1 Mg C ha^{-1} $year^{-1}$ (Bowden et al. 2000) and total soil N (to 60 cm) is approximately 10,000 kg N ha^{-1} (Bowden et al. 1996).

The site is at an elevation of 390 m and has a gentle slope (approximately 5%). The soils have a silty-loam texture and are moderately well drained with a bulk density of 0.52±0.01 (mean±SE) g cm^{-3}, pH (2:1, water/soil) of 4.0 and a cation exchange capacity of 3.73 $cmol_c$ kg^{-1} in the upper 15 cm of the mineral soil. Agriculture was practiced on the site; however, soil profile data suggest that the site was never plowed though it may have been used as a pasture or woodlot (Bowden et al. 2000).

Three control and three N-amended 15×15-m plots were established in 1994. N-amended plots received NH_4NO_3 in six applications from May through October for a total annual rate of 100 kg N ha^{-1}. For most years, N was applied in liquid form with a backpack sprayer, although N was applied in pellet form for several years.

Soil collection and preparation

Soils were collected in late summer from three control and three N-amended plots at Bousson (26 August 2002). After surface litter was removed, organic soil (Oe+Oa, depth of 1–3 cm) was collected from three 20×20-cm areas within each plot. Mineral soils were collected by coring to a depth of 25 cm within the center of each of the 0.04-m^2 areas. Cores were subdivided into 0- to 5-, 5- to 10-, and 10- to 20-cm depths. Depths 0–5- and 5–10-cm were in the A horizon while the 10- to 20-cm depth was in the lower part of the A and into the upper part of the B horizon. Three or four cores were collected from each 0.04-m^2 area and were pooled by depth in the field to obtain sufficient soil for analyses. After passing soil through a 4.0-mm sieve, five replicate subsamples were removed for determination of inorganic N pools, N mineralization and nitrification rates.

The remaining samples were stored in insulated coolers equipped with ice packs and transported back to the Ecosystems Center (Massachusetts) within 36 h. Soil moisture and water holding capacities (WHC) were determined for each depth (Gardner 1982). Samples were stored at +5°C for 3.5 days until CH_4 consumption rates at field soil temperature and moistures were measured. Soils were adjusted uniformly to 30% WHC 24 h prior to determination of CH_4 uptake kinetics.

Analysis of soil net N mineralization and net nitrification rates

Soil NO_2^-+NO_3^- and NH_4^+ pools were determined by extraction with 1 M KCl for 48 h. Concentrations were determined with a Lachat Quick Chem AE automated ion analyzer using a phenate method for NH_4^+ and a hydrazin-reduction method for NO_2^-+NO_3^- (Lachat Instruments 1994).

Laboratory net N mineralization and net nitrification rates were estimated by incubating soil samples aerobically for 10 days at 25°C and inorganic N concentrations were determined as reported above. Rates of N mineralization and nitrification were calculated by the differences between final and initial extractable NH_4^+ and NO_3^-+NO_2^-.

CH_4 chamber fluxes

Chamber anchors were installed in each of the three control and fertilized plots at Bousson and three in each

treatment at Harvard Forest at least 1 month prior to making flux measurements (Steudler et al. 1989; Bowden et al. 1990). CH_4 consumption rates were measured at midday on 26 August 2002 using static chambers. Chamber incubations were initiated by placing the chamber top on the anchor and taking 10-ml headspace air samples at 0, 5, 10 and 20 min using gas-tight Becton-Dickinson syringes. Syringes were transported back to Woods Hole, Massachusetts and analyzed for CH_4 within 36 h of collection by gas chromatography (Steudler et al. 1989). Calibration was accomplished using two certified CH_4 standards (Scott Specialty Gases, Plainfield, N.J.) of 0.601 and 4.08 μl l^{-1} CH_4 in N_2. CH_4 fluxes were calculated using the initial linear change in CH_4 concentration against incubation time. A minimum of three points was used for the calculation of each flux rate. Air and soil temperatures at the surface, 2- and 5-cm depths were measured for each treatment.

Soil CH_4 profiles

CH_4 concentrations were measured at the atmosphere–litter layer boundary and at 5-, 10- and 20-cm depths below the litter layer. This was accomplished using appropriate lengths of 6-mm-outer diameter stainless steel tubes that were sealed at one end and equipped with a two-way stopcock at the other end. Small holes (2 mm diameter) located above the sealed end allowed soil air to diffuse into the tubes. Tubes were installed 1 month prior to collection at Bousson and at least 6 months prior to collection at Harvard Forest. Ten milliliters of soil air was drawn from the tubes 24 h before sampling. Tubes were then allowed to equilibrate with soil air before taking 10-ml samples with gas-tight Becton-Dickinson syringes at the same time as the chamber measurements.

CH_4 consumption under field temperature and moisture conditions

Soils from the organic and 0- to 5-, 5- to 10-, and 10- to 20-cm depths in the mineral horizons from the control and N-amended plots were assayed approximately 3.5 days after soil collection for ambient CH_4 consumption at field temperature and moisture conditions. Twenty grams (dry weight equivalent) was placed in 150-ml Pyrex beakers and stored in plastic bins. Deionized H_2O-saturated absorbent pads were placed in the bottom of the bins to maintain humidity levels and adequate ambient air exchange was provided. Soils were stored overnight at 20°C in a temperature-controlled growth chamber. On the day of the assay, soils were removed from the bins and each beaker placed into a gas-tight 490-ml screw cap mason jar equipped with a stopcock in the lid to sample the jar headspace. Three replicate samples from each plot and soil depth were incubated at 20°C in the dark. Soils were incubated for 3 h with 8-ml headspace gas samples removed every half hour. Headspace CH_4 concentrations were measured by gas chromatography as described above. CH_4 uptake rates were obtained by curve fitting the CH_4 concentration against time to a kinetic decay function and calculating the minimum first derivative of that function. This process was accomplished using the TableCurve software package (Systat 2002).

CH_4 uptake kinetics

Control and N-amended soil samples were assayed for CH_4 uptake kinetics after approximately 7 and 8 days, respectively, from the time of sample collection. Twenty grams (dry weight equivalent) of soil at 30% WHC was placed in 125-ml plastic cups and stored in plastic bins. Deionized H_2O-saturated absorbent pads were placed at the bottom of the bins to maintain humidity levels and adequate ambient air exchange was provided. Samples were stored overnight at 20°C in a temperature-controlled growth chamber. On the day of the experiment, soils were removed from the bins and each cup placed into a gas-tight 490-ml screw cap mason jar equipped with a stopcock in the lid to sample the jar headspace. Headspace CH_4 concentrations were adjusted by adding the appropriate volumes of a 942 μl l^{-1} standard in air (Scott Specialty Gases) to approximately 1.8 μl l^{-1} (no CH_4 added), 5, 10, 15, 20, 30 μl l^{-1}. Three replicate samples from each plot and soil depth were incubated at 20°C in the dark. Control samples were incubated for a total of 4 h with 8 ml of headspace gas samples taken every hour. N-amended samples were incubated for 6 h with hourly sampling of the headspace. CH_4 concentrations were determined by gas chromatography as described above. CH_4 uptake rates were obtained by curve fitting CH_4 concentration against time to a kinetic decay function and calculating the minimum first derivative of that function using TableCurve software package (Systat 2002). Kinetic parameters [$K_{m(app)}$, $V_{max(app)}$] were estimated by nonlinear regression (least squares) of each uptake rate series (1.8, 5, 10, 15, 20, 30 μl l^{-1}) against its midpoint CH_4 concentration using the Michaelis–Menton equation as the model. We used the enzyme kinetics analysis macro within the SigmaPlot software package (SPSS 2001) to accomplish this task. Results from triplicate samples were averaged to obtain a $K_{m(app)}$ and $V_{max(app)}$ for each treatment along with the corresponding SEs.

Statistical analysis

Unless otherwise noted, statistical data analysis was accomplished by using one-way ANOVA (SigmaStat, SPSS 1997). Differences were considered significant when $P<0.05$.

Results and discussion

N dynamics

NH_4^+ concentrations decreased with depth in both treatments (Fig. 1a). Minor differences were seen in the 0- to 5- and 5- to 10-cm depth soils where the control soils had higher NH_4^+ concentrations than the N-amended soil.

A similar pattern was observed for NO_3^-+NO_2^- where concentrations decreased with depth in both treatments (Fig. 1b). The N-amended soils had a slightly higher NO_3^-+NO_2^- concentrations than their corresponding control. The 10- to 20-cm depth soil had a significantly greater concentration of NO_3^-+NO_2^- compared to the control.

Net N mineralization and net nitrification rates were very low, fluctuating between −1 and 1 μg N g^{-1} dry soil day^{-1} with no differences between treatments (Fig. 1c, d). Bowden et al. (2000) reported that a large amount of N mineralization and nitrification at this site occurs during the late spring, which may explain our low rates of mineralization and nitrification because soils were sampled in late summer (August).

CH_4 dynamics

CH_4 concentrations at all depths in the Bousson soil profiles were similar in both treatments, but were reduced by approximately 38% between the soil surface and 5-cm depth (Fig. 2a). Maximum CH_4 concentration (~1.8 μl l^{-1}) was at the surface of the organic soils and reached a minimum value of about 0.5 μl l^{-1} by the 10- or 20-cm depths. Similarly Castro et al. (1994a) observed a decrease from a surface concentration of 1.72 to 0.14 μl l^{-1} for depths below 20 cm in the >50-year-old mixed hardwood control plot at the Harvard Forest long-term N-addition experiment (Magill et al. 1997, 2000) located in central Massachusetts (42°32′N, 72°10′W). Our September 2002 (Fig. 2b) profile data from the same Harvard Forest plots after 14 years of N additions indicated similar patterns as shown by Castro et al. (1994a) for the control plot. However, the Harvard Forest high-N-amended (150 kg N ha^{-1} $year^{-1}$) hardwood soils showed higher concentrations at all depths than the control soils. In fact, the Bousson CH_4 profile for both control and N-amended plots lies between the Harvard Forest low (50 kg N ha^{-1} $year^{-1}$) and high N

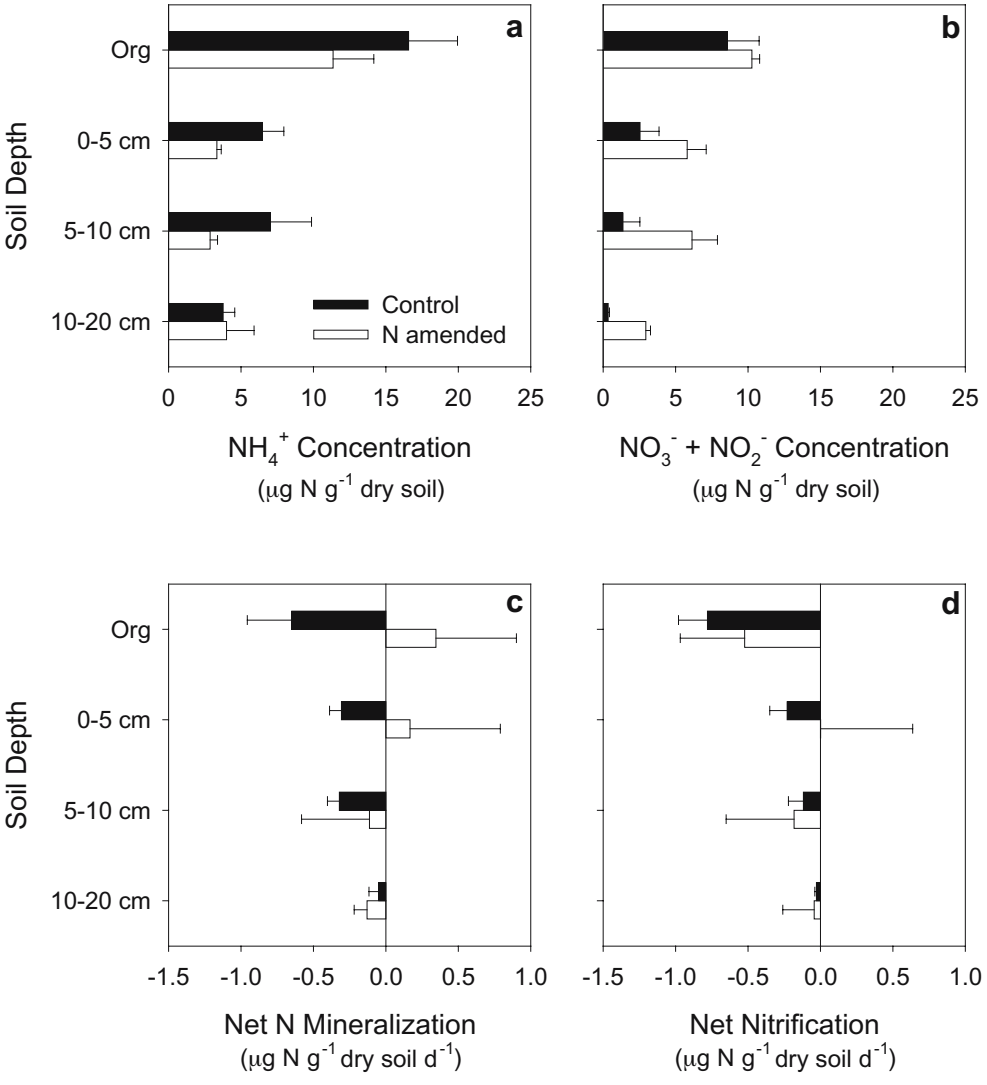

Fig. 1a–d Extractable soil NH_4^+, NO_3^-+NO_2^- concentrations and net N mineralization and nitrification rates determined in a 10-day (*d*) laboratory incubation for control and N-amended soils. *Negative* N mineralization and nitrification rates indicate net immobilization. *Org* Organic horizon

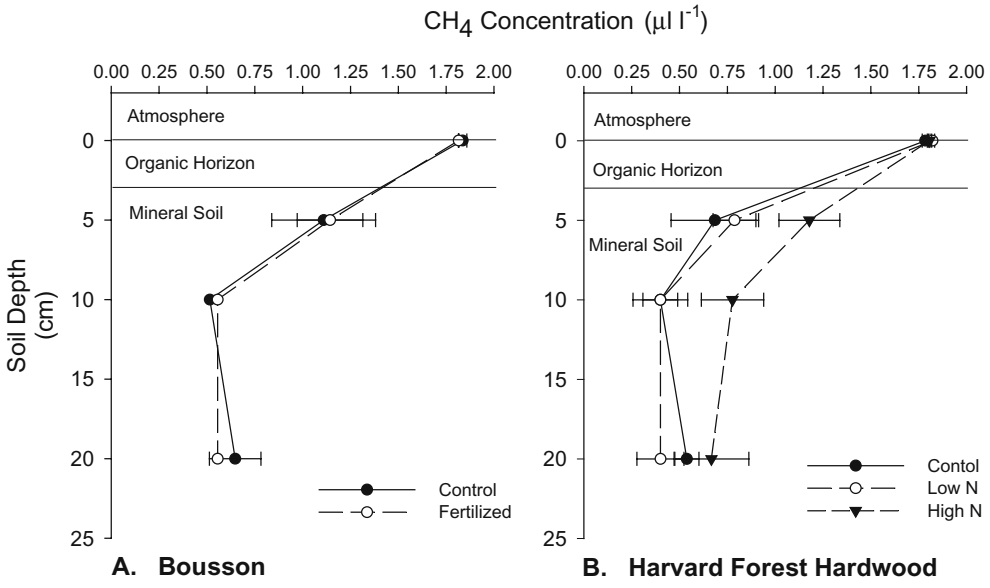

Fig. 2a, b Comparison of soil CH_4 profiles from the Bousson, Pennsylvania forest and Harvard Forest, Massachusetts. *Symbols* represent mean CH_4 concentrations ±1 SD (n=2)

profiles (Fig. 2b). Bousson control soils reduced CH_4 concentrations by approximately 21% in the organic horizon and 38% at 5 cm, compared to reductions at the Harvard Forest of 37 and 63% in the organic and 5-cm soils, respectively. This suggests that Bousson control soils may have less capacity to consume atmospheric CH_4 than Harvard forest soils.

Chamber measurements always showed net CH_4 consumption with rates of 0.19±0.02 (mean±SE) and 0.12±0.01 mg CH_4–C m^{-2} h^{-1} for the control and N-amended treatments, respectively. Rates in the N-amended plots were 35% less (P=0.043, t-test) than the controls. Our single set of chamber measurements showed slightly less CH_4 consumption in the N-amended plots compared to the rate observed in August 1994 (3 months after the beginning of N amendments) at this same site (Bowden et al. 2000). At that time, there was a 28% decrease in the August measurement and an overall annual 24% decrease in CH_4 uptake in the N-amended soils. Castro et al. (1995) also reported that CH_4 inhibition continued to increase in the Harvard Forest N treatments since the onset of N amendments. The annual rate of CH_4 consumption in the N-addition hardwood plots was 23% lower in the low- and high-N-treated plots at the beginning of their measurements, but was 27 and 36% lower in the low-N and high-N plots at the end of their measurement period. Our measurements in September 2002 showed that the degree of inhibition had increased to 40% (low N) and 60% (high N), respectively. The pattern observed at the Harvard Forest N-treated plots indicates that the N-amended Bousson soils may not have reached their maximum degree of CH_4 inhibition. Further inhibition of CH_4 consumption may become evident in future monitoring as N inputs continue.

To assess CH_4 consumption rates throughout the soil column, soils from the segmented cores (organic, 0- to 5-, 5- to 10- and 10- to 20-cm mineral soil depths) were incubated in the laboratory at ambient CH_4 concentration (~1.8 µl l^{-1}), and at field temperature and moistures. The greatest rates were measured in the organic horizon for both the control (0.28±0.03 nmol g^{-1} dry soil h^{-1}) and N-amended (0.16±0.03 nmol g^{-1} dry soil h^{-1}) soils (Fig. 3). In the control, rates in the 0- to 5-cm depth were 65% (P<0.05) less than the organic horizon. Other studies have observed some consumption in the organic horizon, but always at a much lower rate than the upper mineral soil (Yavitt et al. 1995; Saari et al. 1997; Bradford et al. 2001; Wang and Ineson 2003; Gulledge et al. 2004). Maximum CH_4 consumption activity is usually measured in the upper portion of the mineral soil (Whalen et al. 1992; Adamsen and King 1993; Koschorreck and Conrad 1993; Schnell and King 1994; Conrad 1996; Gulledge et al. 1997, 2004). Ours is the first published study of which we are aware

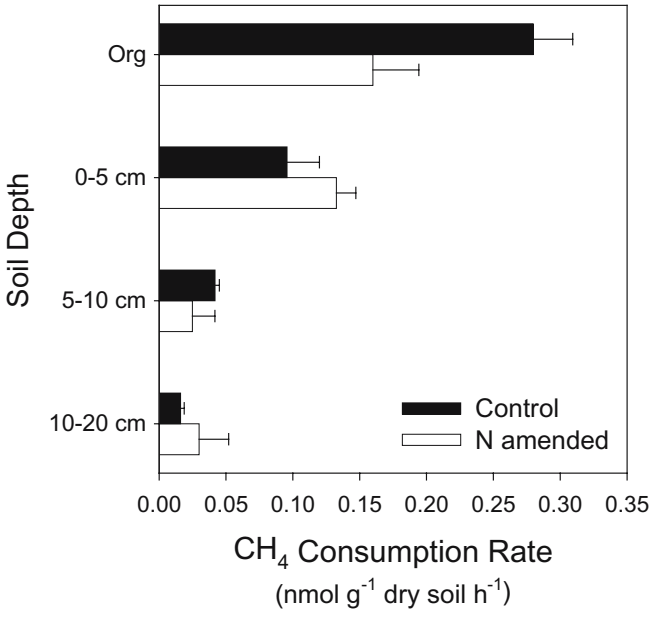

Fig. 3 Atmospheric CH_4 consumption rates in control and N-amended soils from different depths. Ambient oxidation rates were obtained by exposing soils to 1.8 µl l^{-1} CH_4. *Bars* represent mean CH_4 uptake fluxes ±1 SE (n=3)

that reports maximum CH_4 consumption occurred in the organic horizon of the soil profile.

The effect of N addition on CH_4 consumption was observed only in the organic horizon where consumption was reduced significantly (42%) compared to the control (Fig. 3). Rates in the 5- to 10- and 10- to 20-cm depths of the N-amended soils were approximately 81% lower ($P<0.05$) than those of the organic horizon or 0- to 5-cm depth mineral soil.

Gulledge et al. (2004) also found that after 10 years of N addition the rate of CH_4 consumption in organic horizon of the Harvard Forest high-N hardwood stand was reduced significantly (92%) compared to the corresponding control. However, in contrast to the results observed at Bousson, they also saw significant declines in consumption rates for the 0- to 5-cm (62%) and 5- to 10-cm (16%) depths. These results suggest that the Harvard Forest soils may be more susceptible to N additions than those at Bousson because of differences in their N cycles. One explanation for the difference may be due to large differences in the nitrification rates between Bousson and Harvard Forest; Harvard Forest control soils nitrify 1 or 2% of the annual rate of N mineralization (Magill et al. 1997, 2000) whereas Bousson control soils nitrify over 85% of the N mineralized (Bowden et al. 2000). Steudler et al. (1996) found a pattern of lower CH_4 consumption rates in a variety of forest soils with high rates of nitrification. They reasoned that increases in rates of nitrification indicate the extent to which NH_4^+ is biologically available to nitrifiers and might therefore indicate the extent to which other soil microbes, including CH_4 consumers, are exposed to NH_4^+. Thus, additions of NH_4^+ to the Harvard Forest soils cause a much greater relative increase in the available NH_4^+ pool that the CH_4 consumers are exposed to than in the Bousson soils and result in a larger decrease in the CH_4 consumption rates. Another possibility may be that there are differences in the population size and/or composition of consumers at the two locations.

The large decrease in consumption in the Bousson organic horizon of the N-amended plots was the major contributor to the overall 35% decrease in the chamber-based CH_4 consumption rates compared to the control. Our results are consistent with the results of Gulledge et al. (2004) who also found that N additions dramatically decreased CH_4 consumption in the organic horizons at the Harvard Forest sites. The location of the decrease suggests that the N status of the organic horizon may be an important factor controlling the overall capacity of this forest and perhaps other temperate forests to consume CH_4. While our laboratory mineralization and nitrification analyses (Fig. 1) did not reveal important differences between the control and N-amended treatments in the organic horizon, Bowden et al. (2000) reported that a large amount of annual N mineralization and nitrification occurs during the late spring, with much lower rates in late summer. Hence, it is not surprising that we did not see major N-cycling differences at the time of our study.

CH_4 uptake kinetics

All soil incubations showed typical Michaelis–Menten saturation kinetics for CH_4 uptake. Generally, $K_{m(app)}$ values of the control soils remained constant with depth except in the 5- to 10-cm depth where $K_{m(app)}$ increased by 177% ($P<0.05$) compared to the organic horizon or 0- to 5-cm depth (Fig. 4a). This increase may indicate that the 5- to 10-cm depth in the control treatment could periodically be exposed to higher concentrations of CH_4 perhaps due to methanogenesis at times when soils are

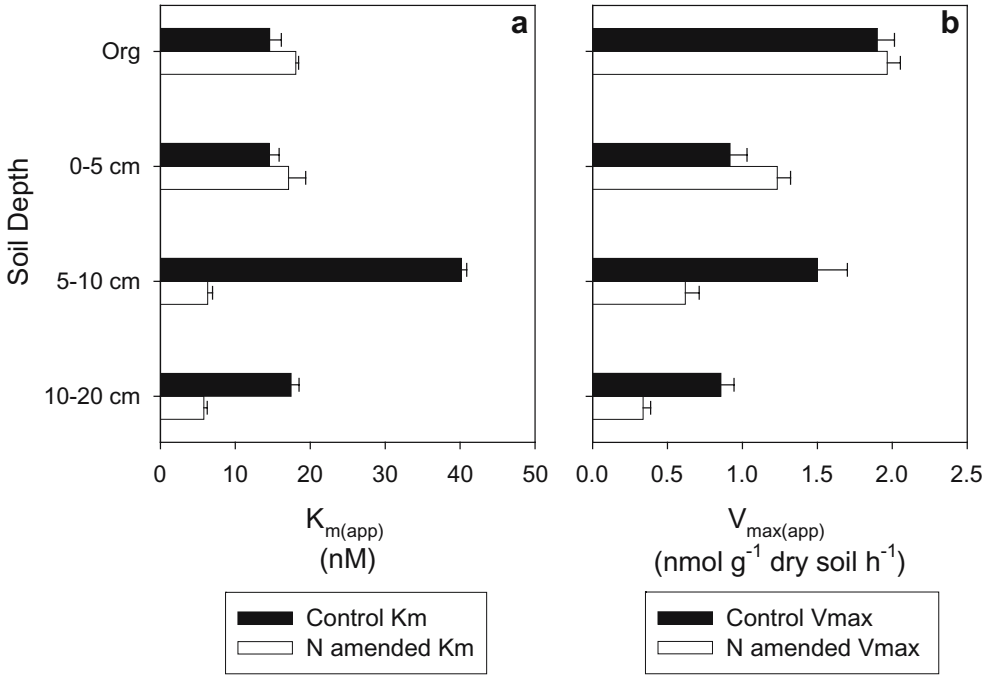

Fig. 4a, b Michaelis–Menten kinetic parameters for CH_4 uptake in control and N-amended soils from different depths. *Bars* represent mean apparent (*app*) K_m and V_{max} values ±1 SE (n=3)

extremely wet. These soils, which have a high silt content, have routinely been observed to be near or at saturation from late autumn until late spring, and methanogenic and methanotrophic bacterial communities might well be driven by these soil moisture conditions. Additionally, N deposition may have also affected primarily the organic horizon and 0- to 5-cm depth, hence the dramatic change in the $K_{m(app)}$ and $V_{max(app)}$ values below 5-cm depth (Fig. 4a, b).

There are few studies that report kinetic values in forest stands for both the organic and upper mineral soils. In two studies that have, a decreasing or equal $K_{m(app)}$ value with depth was observed (Bradford et al. 2001; Gulledge et al. 2004). Literature values of $K_{m(app)}$ for the organic horizon generally ranged from 42 to 81 nM, and from 5.5 to 69 nM for upper mineral soils (Bradford et al. 2001; Knief et al. 2003; Gulledge et al. 2004). Our $K_{m(app)}$ values for the organic horizon and the 0- to 5-cm mineral soil in the control were generally 2–4 times lower than values reported by Gulledge et al. (2004). The higher values reported by Gulledge et al. (2004) may be the result of lower N deposition over time at the Harvard Forest. Estimates using 1994–2003 data from the National Atmospheric Deposition Program (NAPD), National Trends Network (http://nadp.sws.uiuc.edu/) show that the Bousson site received an average of approximately 7.6 kg N ha^{-1} $year^{-1}$ or 36% more wet N deposition (NH_4^++NO_3^-) and approximately 3.1 kg NH_4 ha^{-1} $year^{-1}$ or 41% more wet NH_4^+ deposition from 1994 up to and including 2003 than in the Harvard Forest region. Our $K_{m(app)}$ value for the 0- to 5-cm depth is consistent with those reported for upper mineral soils from mixed deciduous forests in Germany (Knief et al. 2003). These forests have received even higher rates of wet N deposition, up to 18 kg N ha^{-1} $year^{-1}$, for more than four decades.

In the Bousson N-amended soils, $K_{m(app)}$ values in the organic horizon and 0- to 5-cm mineral soil were similar (Fig. 4a). This is in contrast to the results of Gulledge et al. (2004) who found more than a threefold decrease in the $K_{m(app)}$ values between these horizons. Furthermore, in our study, repeated N additions did not decrease the $K_{m(app)}$ values of the most active CH_4-consuming soils (organic horizon and 0- to 5-cm depths) compared to the controls. A plausible explanation is that because of long-term increased N deposition at our site (Bousson surface soils), organic and 0- to 5-cm depth may have already reached minimum $K_{m(app)}$ values, whereas the Harvard Forest soils have not. However, there was a significant decrease in $K_{m(app)}$ values for N-amended soils below 5-cm depth. It may be that the effect of N deposition only extends to the organic and 0- to 5-cm depth soils so that the additional N reaches deeper into the soil profile. This is reflected in the significant decrease in $K_{m(app)}$ and $V_{max(app)}$ values in the soils below 5 cm.

Our $V_{max(app)}$ values for the Bousson control soils were lower than those reported by Gulledge et al. (2004) in their hardwood stand, but were similar to values reported by Knief et al. (2003) for the mineral horizons of their mixed and deciduous forests in Germany (Fig. 4b). We observed no N effect on $V_{max(app)}$ in the organic horizon or the 0- to 5-cm mineral soil, but did observe a decrease of 60–67% below 5 cm. Gulledge et al. (2004) generally observed a N effect on $V_{max(app)}$ for all depths with the greatest decreases (83–89%) in the organic horizon and the 0- to 5-cm mineral soil. The higher $V_{max(app)}$ values and $V_{max(app)}$ differences along the profile reported by Gulledge et al. (2004) may be the result of lower long-term N deposition at the Harvard Forest.

Conclusions

This investigation revealed several long-term consequences of N deposition and N additions on CH_4 dynamics in a productive temperate deciduous forest. Our study is the first to find that the maximum rate of CH_4 consumption occurred in organic horizon material of the soil profile. The reduced rate of CH_4 consumption in the organic horizon was the major contributor to the 35% decrease in chamber CH_4 consumption in the N-amended soils. Hence, the organic horizon N status of this ecosystem plays a key role in the regulation of atmospheric CH_4 consumption. Bousson surface soils (organic horizon and 0- to 5-cm mineral horizon), which receive higher N inputs from deposition than Harvard Forest, had less capacity to consume atmospheric CH_4 than Harvard Forest surface soils. The kinetic parameters, $K_{m(app)}$ and $V_{max(app)}$ values for the Bousson control soils were much lower than the Harvard Forest hardwood control soil values but similar to values reported for upper mineral soils from mixed deciduous forests in Germany that receive high N deposition. Adding an additional 100 kg N ha^{-1} $year^{-1}$ for 8 years to the Bousson forest had a minimal effect on the kinetic parameters in the organic horizon and 0- to 5-cm mineral soil. This may be because these horizons have already reached the maximum effect with respect to N and the additional N reached deeper into the soil profile. This is reflected in the significant decrease in $K_{m(app)}$ and $V_{max(app)}$ values in the soils below 5-cm depth. These findings imply that continued long-term N additions to soils from both deposition and N amendments may have altered the size, composition and/or the physiology of the CH_4-consuming organisms.

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