FACTORS REGULATING NET METHANE FLUX IN URBAN FORESTS AND GRASSLANDS

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Abstract. Methane is a potent greenhouse gas consumed through a biological oxidation process in soils. During urban and suburban land use change, native ecosystems are shifted to impervious surfaces, urban forests, and grasslands (lawns). These changes can influence CH_4 uptake through alterations in physical, chemical and biological soil conditions. A recent study in the Baltimore, MD USA metropolitan area found high net CH_4 uptake in rural forests, lower net uptake in urban forests and complete inhibition of uptake in urban lawns. In this study, we investigated four factors that could be causing this inhibition; reduced diffusion of methane into soils, production of methane in anaerobic microsites, inhibition of uptake by nitrogen additions from fertilizer or atmospheric deposition, and the presence of an unknown inhibitor in urban soils. Soil samples (three replicates) were collected from four rural forest plots, four urban forest plots, and four urban grassland plots (36 samples total). Methane consumption rates were measured under ambient atmospheric and 10 ppm CH_4 levels, with and without additions of 5 mg N/kg as NH_4SO_4. CH_4 production rates were measured under anaerobic conditions. Patterns of consumption in sieved soils in the laboratory were comparable to previous field results, eliminating diffusion as a possible inhibiting mechanism. No methane was produced in anaerobic incubations, signifying that microsite production was not important. N had no effect on consumption, suggesting that N inputs do not immediately inhibit uptake. However, grasslands and urban forests had higher rates of N cycling by nitrification and mineralization, and there were strong negative relationships between CH_4 uptake and nitrification, suggesting that long-term differences in N cycling associated with urban land use change may have led to a reduction in the microbial populations responsible for methane uptake.

INTRODUCTION

Atmospheric methane (CH_4) is a powerful greenhouse gas, 25 times more potent than $C0_2$ and is considered to contribute 15-20% to atmospheric global warming (IPCC, 2001). The CH_4 atmospheric concentration has increased 151% since 1750 as a result of anthropogenic factors causing an imbalance between methane sources and sinks (IPCC, 2001). The primary natural source of CH_4 is wetlands that support anaerobic soil conditions that allow for CH_4 production. The largest biological sink of CH_4 is aerobic upland soil, which accounts for 6-10% of all CH_4 sinks, or about 30 Tg CH_4 yr^{-1} (IPCC, 2001; Menyailo et al., 2008).

The net flux of CH_4 from soils is the result of the anaerobic (production) and aerobic (consumption) biological processes (Wachinger et al., 2000). Upland aerobic soils tend to have a higher uptake (consumption) than production rate, making these soils methane sinks (Wachinger et al., 2000). Aerobic bacteria that live in non-saturated soils, such as forest, grassland and desert soils, consume CH_4 as an energy source. The consumption of methane by these methanotrophs, is the result of an entirely biological oxidation process and is regulated by a variety of environmental factors, such as soil temperature, moisture, nitrogen content, organic matter content, and pH (Ridgewell et al., 1999). The primary control of CH_4 consumption however is the rate of diffusion of methane into the soil, which is necessary for the flow of methane from the atmosphere to methanotrophs (Born et al., 1990; Ridgewell et al., 1999). Higher rates of diffusion are found in coarser and drier soils, making soil texture and moisture highly influential on CH_4 consumption (Castro et al., 1995; Smith et al., 2000; Groffman et al., 2006). Methane producers are methanogenic bacteria that convert CO_2 into methane through a metabolic process in anaerobic environments (Megonigal and Guenther, 2008).

Land-use change may have severe consequences for the soil methanotroph and methanogen communities, affecting the strength of the soil methane sink and altering the global CH_4 net flux (Moiser et al., 1991; Reay and Nedwell, 2004; Menyailo et al., 2008). While many studies have examined the effects of land use changes on methane cycling (Moiser et al., 1991; Ojima et al., 1993; Goldman et al., 1995; Wang and Bettany et al., 1997; Menyailo et al., 2008) there is considerable uncertainty about the mechanisms underlying these effects.

Urbanization is one of the most profound land-use changes occurring globally, with considerable consequences for ecosystem services (Kaye et al., 2006). Urban land-use change can influence CH_4 uptake through environmental alterations in chemistry and climate, as well as through the transformation of native ecosystems into more human-dominated systems (Groffman and Pouyat, 2009). Goldman et al. (1995) found that CH_4 uptake rates in forests in the urban center of New York City were low relative to rates in rural forests, and similar results were found in forests in the Baltimore metropolitan area (Groffman et al. 2006) suggesting that there is an urban atmospheric effect on this process. Groffman and Pouyat (2009) also observed low uptake in urban forests relative to rural forests in Baltimore, but reported almost complete inhibition of uptake in urban grasslands (lawns). Urban grasslands are defined as "ecosystems dominated by turf-forming species created and maintained by humans for aesthetic and recreational (not grazing) purposes (Groffman et al. 2009)." There are over 150,000 km^2 of urban grasslands in the United States and they cover approximately 10% of the state of Maryland (Milesi et al., 2005).

The objective of this study was to determine what factors underlie the urban atmospheric and land conversion effects on CH_4 uptake reported by Goldman et al. (1995), Groffman et al. (2006) and Groffman and Pouyat (2009). We hypothesized that four factors could play a role: (1) reduced diffusion of methane into urban soils, (2) increased production of methane in anaerobic microsites in urban soils, (3) inhibition of uptake by nitrogen additions from fertilizer or atmospheric deposition, and (4) and the presence of an unknown inhibitor in urban soils. We evaluated these factors by sampling the rural forest, urban forest, and urban grassland soils in the Baltimore metropolitan area previously studied by Groffman et al. (2006) and Groffman and Pouyat (2009) and conducting a series of laboratory incubations under different conditions to isolate the different factors. Any possible diffusion effects were eliminated by incubating sieved soils in jars; anaerobic microsites were tested for by incubating soils anaerobically; short-term nitrogen effects were tested by additions of inorganic N; long-term nitrogen effects were evaluated by measuring rates of potential net N mineralization and nitrification; and the presence of inhibitory compounds was tested by incubating mixtures of urban and rural soils and looking for disproportionate reductions in uptake.

METHODS

Study sites

The urban and rural forest and urban grassland plots have been extensively described in Groffman et al., (2006, 2009) and are part of a network of long-term study plots established by the National Science Foundation funded Baltimore urban LTER project (BES) in Baltimore City and Baltimore County, Maryland (76°30', 39°15'). The forest plots were dominated by tulip poplar (*Liriodendron tulipifera*) and oaks, primarily chestnut (*Quercus prinus*), scarlet (*Quercus coccinea*) and white (*Quercus alba*). Urban grasslands in the region are dominated by Kentucky bluegrass (*Poa* pratensis), tall fescue (*Festuca arundinacea spp.*), fine fescue (*Festuca spp*), and white clover (*Trifolium repens).* Average annual precipitation is approximately 1060 mm y^{-1} and stream discharge is approximately 380 mm y^{-1} (Doheny, 1999). Atmospheric N deposition in the Baltimore metropolitan area is estimated at 1.1 g N m^{-2} y^{-1} (Groffman et al., 2004)

The BES network of long-term study plots consists of eight forested and four grass plots. The forest plots were established in 1998 in remnant forests in Baltimore City and County parks. The grass plots were established in 2001 and represent a range of management intensities from unfertilized, infrequently mowed plots to plots with high inputs of fertilizer and herbicides and frequent mowing. These are "institutional lawns" on the campuses of

a secondary school and a University and have been managed in the same way for more than 10 years. Clippings were left in place on all grass plots.

Soil assays

Soil samples (three replicates) were taken in June 2009 to 10 cm depth and were placed in plastic sealed bags (36 samples total) and then stored at 4°C at field moisture between sampling and analysis.

Soil samples were hand sorted and mixed and rocks and large roots were removed. Soil moisture content was determined by drying at 60°C for 48 hours. Soil organic matter content was determined through loss on ignition by drying at 450°C for 4 hours.

Concentrations of ammonium (NH_4^+) and nitrate (NO_3^-) in the soil were determined by extraction with 2M KCl solution followed by colorometric analysis on a flow injection analyzer. Additional soil samples were incubated for 10 days in glass quart mason jars fitted with airtight lids and butyl rubber septa to allow for gas sampling. After incubation, gas samples were taken with a syringe and analyzed for CO_2 by gas chromatography. Final concentrations of NH_4^+-N and NO_3^-N were determined by 2M KCl extraction as described above. Potential net N mineralization was calculated from the accumulation of NH_4^+ plus NO_3^-, potential net nitrification was calculated from the accumulation of NO_3^-, and microbial respiration was calculated from the accumulation of CO_2 over the 10-day incubation.

CH_4 flux measurements

All CH_4 consumption assays were run in 1 liter mason jars with 20g soil held at field moisture and room temperature that were sampled after 1, 3, and 5 hours of incubation. In addition to consumption of ambient CH_4, consumption was measured in jars with a headspace CH_4 concentration of 10 ppm, achieved with a 7.2 ml injection of 1000ppm CH_4 in N_2 balance at time zero. The effects of inorganic N additions on consumption of both ambient and 10 ppm CH_4 were evaluated by amending soils with 5 mg N/kg as NH_4SO_4 just before incubation.

The CH_4 production anaerobic assay was run in 125 ml Erlenmeyer flasks fitted with Butyl rubber stoppers. The flasks were made anaerobic by repeated evacuation and flushing with N_2 gas and were confirmed anaerobic with BD BBC Dry Anaerobic Indicator Strips. Gas samples were taken after 24 hours of incubation.

The presence of an inhibitor of CH_4 uptake in urban soils was tested for by preparing 50:50 mixtures of rural forest and urban grassland soil and measuring consumption of ambient levels of CH_4. Disproportionate reductions in uptake rates in the rural forest soils would be taken as evidence of the presence of an inhibitor.

CH_4 levels were measured through direct injection on a Shimadzu gas chromatograph (GC) equipped with a Flame Ionization Detector and ultra high purity helium carrier gas (FID temperature = 140°C). Methane gas was separated from N_2/O_2 with an Alltech Porapak Q80/100 colomn (2m x 1/8" OD x 0.085" ID) at 40°C.

Statistical analysis

Differences between urban forests, rural forests and urban grasslands were testing using one-way analysis of variance, with a Duncan's multiple comparison test. Treatment effects were evaluated using two-way analysis of variance with ecosystem type and treatment as main effects. All analyses were run using the Statistical Analysis System (SAS, 1988).

RESULTS

Soil properties

There were no significant differences in soil moisture or organic matter content across all land use types (Table 1). However, there was significant ($p < 0.05$) variation in mineralization and nitrification rates among the sites. Mineralization was significantly lower in the rural forest than in the urban forest or grassland sites, which did not differ. Nitrification rates were significantly lower in the rural forest than in the urban forest, which in turn were significantly lower than rates in the urban grassland (Figure 1). .

CH_4 Consumption

Consumption of ambient levels of CH_4 was highest in the rural forest followed by the urban forest and the urban grassland which (all differences $p < 0.05$, Figure 2). CH_4 consumption at 10 ppm levels showed the same pattern, but rates were much higher than at ambient levels (Figure 3).

Nitrogen additions had no effect on consumption of either ambient (Figure 4) or 10 ppm (Figure 5) levels of headspace CH_4. No CH_4 production was observed in the anaerobic assay (data not presented).

There was no evidence for the presence of compounds that can inhibit CH_4 uptake in urban soils. A 50:50 mix of rural forest and urban grassland soil had an uptake rate (-0.008 mg kg^{-1} h^{-1}) very close to the mean of the rural forest (-0.023) and urban grassland (0.007) soils.

DISCUSSION

Groffman and Pouyat (2009) reported low CH_4 uptake rates in the field for urban forests relative to rural forests, and observed almost complete inhibition of uptake in urban grasslands. This same variation in CH_4 uptake across the land-use type was found in the laboratory incubations under ambient CH_4 atmospheric concentrations, confirming a major disturbance in the biogeochemical CH_4 cycling of the urban soils in the Baltimore area. It should be noted though that the differences between the rural and urban forest uptake rates are more extreme in the laboratory incubations then in the field rates observed by Groffman and Pouyat (2009). The laboratory incubation CH_4 uptake rates for the rural and urban Baltimore forest soils however are comparable to incubations of rural and urban forest soils in New York City (Goldman et al., 1995). The complete inhibition of CH_4 uptake in the urban grassland soil is much greater then previous studies with reported inhibitory effects in laboratory incubations (Blankinsmith et al., 2010), which have shown a degree of inhibition, but not a complete halt in uptake.

The primary control of CH_4 uptake is the rate of diffusion of atmospheric methane into the soil; diffusion is primarily controlled by soil texture and moisture because a lower pore density and a higher soil moisture content can increase resistance for atmospheric CH_4 transport into the soil (Ridgewell et al., 1999; Groffman et al., 2006; Blankinship et al., 2010). There is no significant difference in the moisture content of the sample sites, indicating that diffusion did not play a role in the decreased CH_4 uptake rates in the urban soils. Additionally, variations in pore densities that may have influenced diffusion in the field were eliminated in the laboratory by homogenization of the soil before incubations. Because the patterns of consumption in the laboratory were comparable to previous field results, it is clear that diffusion is not a possible inhibiting mechanism of CH_4 consumption.

Since the CH_4 net flux from soils is the result of the anaerobic and aerobic biological processes, increased production of methane in upland soils can alter the net flux so that CH_4 uptake appears to be diminished (Wachinger et al., 2000). Methane production in anaerobic microsites has been shown to influence net CH_4 emissions (Wachinger et al., 2000; Blankinship et al., 2010). No methane was produced however in laboratory

anaerobic incubations, so it is apparent that microsite CH_4 production is not a contributing factor in the decreased CH_4 flux for both the urban forest and grassland soils.

N additions have been shown to inhibit CH_4 oxidation in many, but not all, studies through competition with nitrifies, in forest, grassland, cultivated, and urban ecosystems (Moiser et al., 1991; Goldman et al., 1995; Reay and Nedwell, 2004; Blankinship et al., 2010). Nitrogen additions can come from direct inputs of nitrate or ammonium through fertilization, or from increased atmospheric N deposition in urban centers (Groffman and Pouyat, 2009). The mechanisms for N inhibition of CH_4 uptake however are complex and poorly understood, so that the relationship between N cycling and CH_4 are not always obvious (Groffman and Pouyat, 2009; Blankinship et al., 2010).

Ammonium additions had varying effects on CH_4 consumption, with the high-affinity (ambient) CH_4 incubation exhibiting no change in consumption but with the low-affinity CH_4 incubation showing a reduction in consumption across all three land-use types. The findings from this study are comparable to Reay and Nedwell (2004), who found elevated NH_4 concentrations caused a significant effect in low affinity CH_4 oxidizers, but not in high affinity CH_4 oxidizers. Although the different responses of the high-affinity and low-affinity CH_4-consuming bacteria has implications for the different methanotrophic populations, the lack of significant response to N additions in the ambient assay still fails to explain field results. Since N additions had no effect on ambient consumption, the N inputs do not play a role in the short term for the urban forest and grassland plots.

The Baltimore urban grasslands and forest soils had high rates of N cycling from nitrification and mineralization rates, indicating that long-term differences in N cycling associated with maintenance (fertilization) and from atmospheric N deposition that may have led to a reduction in the methane consuming microbial populations. Long-term inputs of fertilizer have been suggested to cause a decrease in methanotrophic populations from niche competition with nitrifiers, implying that the N turnover rate has a greater influence on CH_4 uptake then the total N concentrations (Moiser et al., 1991; Goldman et al., 1995). It is important to note however that the lawns represent a wide range of management techniques, with some sites receiving no fertilizer and others receive large quantities (200 kg N ha-1 year-1) (Groffman and Pouyat, 2009). Additionally, there is no data available on atmospheric deposition for the sites, although it is likely that N deposition is greater in the urban core (Groffman and Pouyat, 2009). These long-term changes may have profound implications for CH_4-consuming communities across urban ecosystems and further research should be conducted.

As presented in Groffman and Pouyat (2009), the inhibition of CH_4 uptake in urban lawns does not appear to have a significant effect on greenhouse gas forcing. The lack of methane consumption however does demonstrate a major shift in the biogeochemical cycling of urban centers, emphasizing how difficult it is to predict and understand urban biogeochemical cycles. The specific cause of the variations in nitrification rates has yet to be indentified, and could be attributed to an unknown urban effect other then fertilization and high N atmospheric deposition. Perhaps more importantly though is the need to identify if these changes in the microbial population can be reversed, and if so, how long it will take.

ACKNOWLEDGEMENTS

This material is based upon work supported by the National Science Foundation under Grant No. DBI 0552871.

Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

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APPENDIX

TABLE 1. Soil moisture and organic matter content and potential net N mineralization and nitrification rates in rural forest, urban forest and urban grassland sites in the Baltimore metropolitan area. Values are mean (standard error) of three samples taken in four replicate sites of each ecosystem type. Values with different superscripts within a row are significantly different at $p < 0.05$ in a one-way analysis of variance with a Duncan's multiple range test.

	Rural Forest	Urban Forest	Urban Grass
Moisture content (%)	36.2[a]	42. 9[a]	30.1[a]
Organic matter (%)	8.2[a]	7.4[a]	7.3[a]
Mineralization (ug-N/g dry soil/day)	0.31[b]	0.61[a]	0.82[a]
Nitrification (ug-N/g dry soil/day)	0.03[c]	0.41[c]	0.84[a]

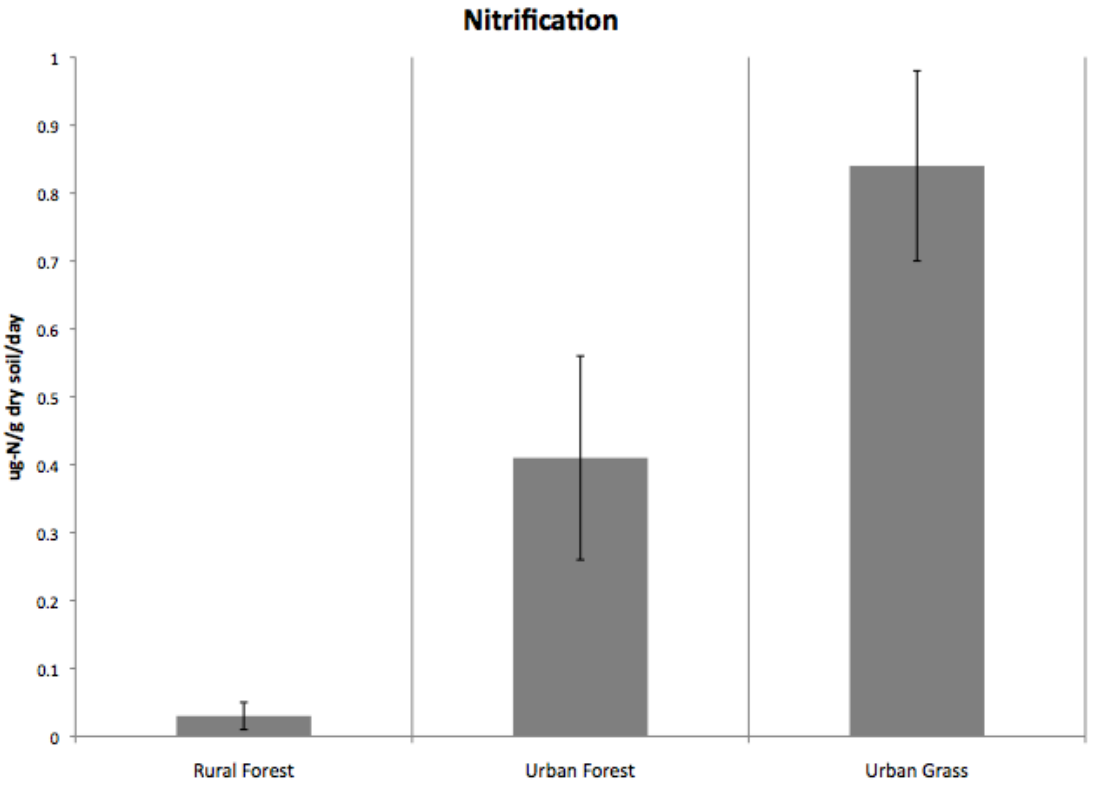

FIGURE 1. Potential net nitrification rates in soils from rural forest, urban forest and urban grassland sites in the Baltimore metropolitan area. Values are mean (standard error) of three samples taken in four replicate sites of each ecosystem type. Bar with different superscripts within a row are significantly different at $p < 0.05$ in a one-way analysis of variance with a Duncan's multiple range test.

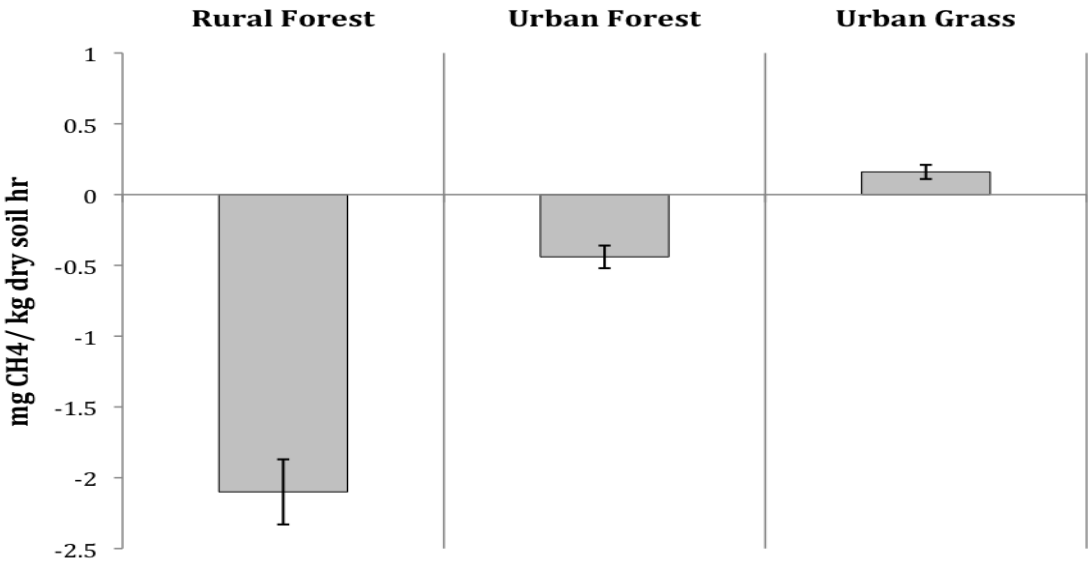

FIGURE 2. Consumption of ambient levels of CH_4 in soils from rural forest, urban forest and urban grassland sites in the Baltimore metropolitan area. Values are mean (standard error) of three samples taken in four replicate sites of each ecosystem type. Bar with different superscripts within a row are significantly different at $p < 0.05$ in a one-way analysis of variance with a Duncan's multiple range test.

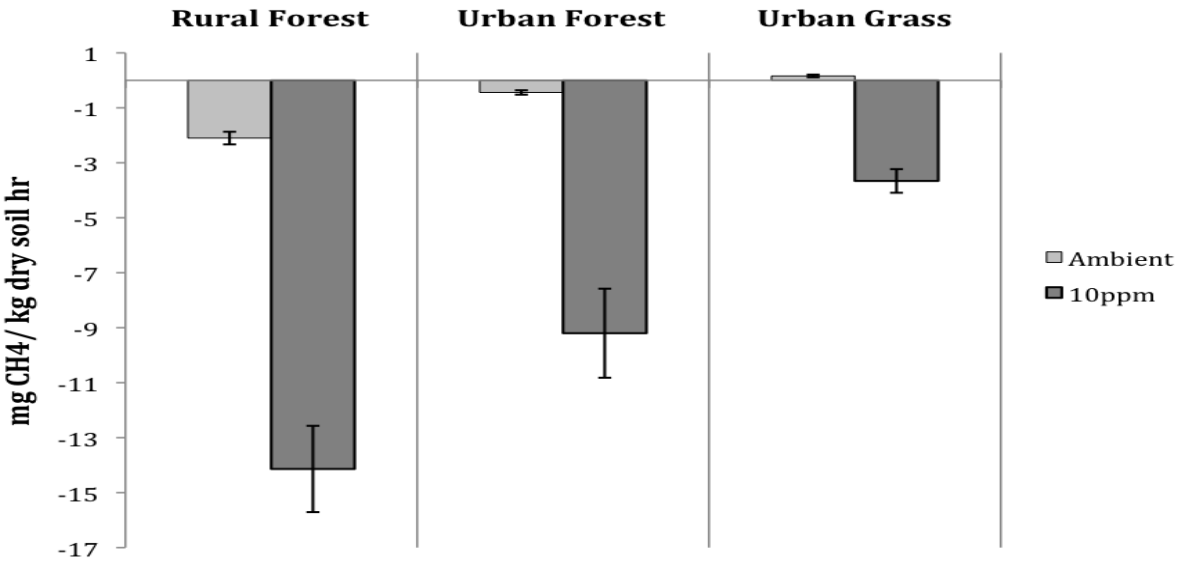

FIGURE 3. Consumption of ambient and 10 ppm levels of CH_4 in soils from rural forest, urban forest and urban grassland sites in the Baltimore metropolitan area. Values are mean (standard error) of three samples taken in four replicate sites of each ecosystem type. Bar with different superscripts within a row are significantly different at $p < 0.05$ in a one-way analysis of variance with a Duncan's multiple range test. Ambient data repeat data from Figure 2 and are included here only for reference.

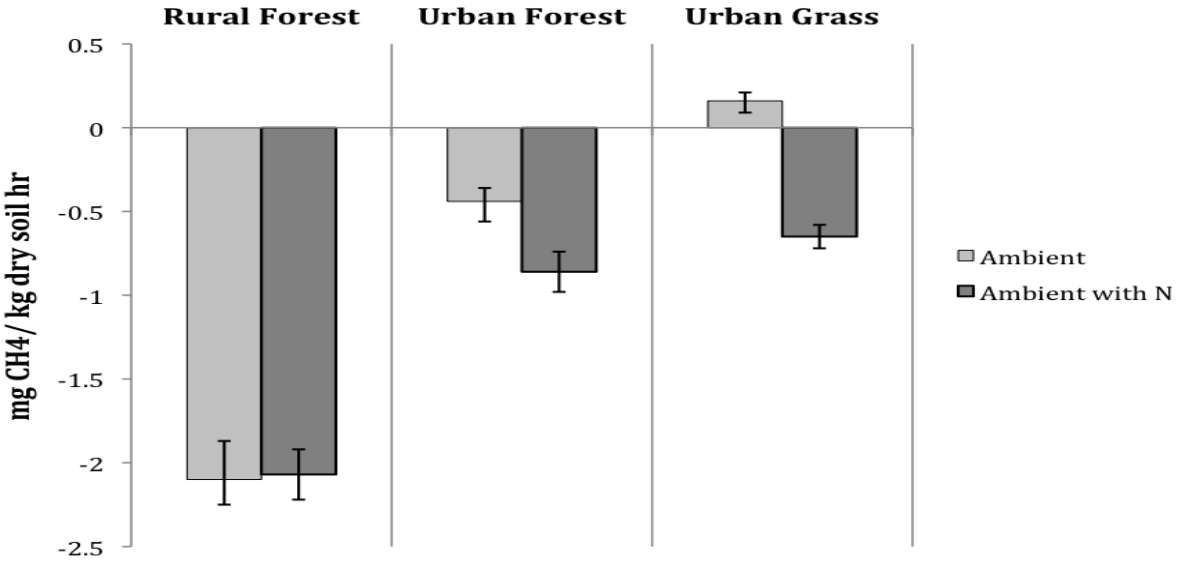

FIGURE 4. Consumption of ambient levels of CH_4 in unamended or N amended soils from rural forest, urban forest and urban grassland sites in the Baltimore metropolitan area. Values are mean (standard error) of three samples taken in four replicate sites of each ecosystem type. Bar with different superscripts within a row are significantly different at $p < 0.05$ in a one-way analysis of variance with a Duncan's multiple range test.

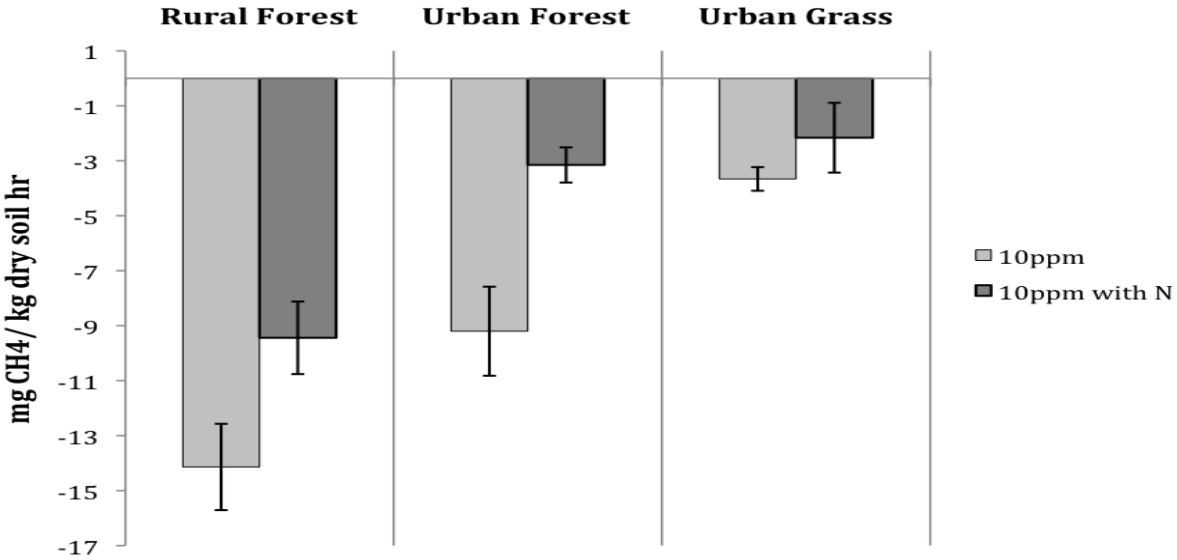

FIGURE 5. Consumption of 10 ppm levels of CH_4 in unamended or N amended soils from rural forest, urban forest and urban grassland sites in the Baltimore metropolitan area. Values are mean (standard error) of three samples taken in four replicate sites of each ecosystem type. Bar with different superscripts within a row are significantly different at $p < 0.05$ in a one-way analysis of variance with a Duncan's multiple range test.