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Methane Uptake in Urban Forests and Lawns

PETER M. GROFFMAN*
Cary Institute of Ecosystem Studies Box AB, Millbrook New York 12545

RICHARD V. POUYAT
U.S. Forest Service, Rosslyn Plaza, Building C, 1601 North Kent Street, fourth Floor, Arlington, Virginia 22209

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The largest natural biological sink for the radiatively active trace gas methane (CH_4) is bacteria in soils that consume CH_4 as an energy and carbon source. This sink has been shown to be sensitive to nitrogen (N) inputs and alterations of soil physical conditions. Given this sensitivity, conversion of native ecosystems to urban, suburban, and exurban managed lawns thus has potential to affect regional CH_4 budgets. We measured CH_4 fluxes monthly from four urban forest, four rural forest and four urban lawn plots in the Baltimore, MD, metropolitan area from 2001 to 2005. Our objectives were to evaluate the effects of urban atmospheric and land use change on CH_4 uptake and the importance of these changes relative to other greenhouse forcings in the urban landscape. Rural forests had a high capacity for CH_4 uptake (1.68 mg m^{-2} day^{-1}). This capacity was reduced in urban forests (0.23 mg m^{-2} day^{-1}) and almost completely eliminated in lawns. Possible mechanisms for these reductions include increases in atmospheric N deposition and CO_2 levels, fertilization of lawns, and alteration of soil physical conditions that influence diffusion. Although conversion of native forests to lawns had dramatic effects on CH_4 uptake, these effects do not appear to be significant to statewide greenhouse gas forcing.

Introduction

Methane (CH_4) is a radiatively important atmospheric trace gas, with a "global warming potential" more than 20 times that of carbon dioxide (CO_2) (*1*). Observed increases in atmospheric CH_4 concentrations over the last several decades could either be caused by increases in the sources or decreases in the sinks of this gas (*2*). Natural sources are dominated by wetlands that support anaerobic soil conditions that foster microbial CH_4 production (*3, 4*). The largest natural biological sink of CH_4 is aerobic bacteria in nonsaturated soils that consume CH_4 as an energy and carbon source (*1, 5*). Temperate forest, grassland, and desert soils have all been shown to be significant sinks for atmospheric CH_4 (*6*−*10*).

Understanding and managing regional and global greenhouse gas budgets involves understanding how diverse natural and anthropogenic factors influence production and consumption of different gases (*11*). For CH_4 production, much work has focused on climate change effects on wetland moisture status and anaerobiosis (*12*). Analysis of factors affecting the ability of drier soils to consume CH_4 has focused on nitrogen (N), as many studies have shown that N additions inhibit CH_4 uptake in both short and long-term studies (*7, 8*). However, there is considerable uncertainty as to the mechanism of this inhibition and its importance over large areas (*13, 14*). Uptake is also affected by physical factors that influence diffusion (*15*), and these are affected by multiple human activities, such as tillage and irrigation (*16*).

Urban, suburban, and exurban land-use change is occurring over large areas of the globe (*17*) with significant effects on multiple ecosystem functions and services, including CH_4 uptake (*18, 19*). In the Chesapeake Bay region of eastern North America, native forests were converted to agricultural use by European settlers beginning in the 18th century and the area was then largely reforested in the first half of the 20th century (*20, 21*). Since 1950, there has been a marked expansion in urban and suburban land-use and the region is now covered by a mix of relict forest, agricultural and urban/suburban lands (*22, 23*).

Urban and suburban land use change has two major aspects; wholesale conversion of native ecosystems to human settlements, and more subtle environmental changes in atmospheric chemistry and climate (*24*). Both these aspects of change can have important effects on CH_4 uptake by altering soil N flows and physical conditions. A major component of the wholesale conversion of native ecosystems to human settlements is the creation of lawns; ecosystems dominated by turf-forming species created and maintained by humans for aesthetic and recreational (not grazing) purposes. Approximately 8% of the land base in the lower 48 United States is in urban land use (*25*), and 41% of this area is classified as residential, most of which is made up of lawns (*26, 27*). The total estimated area of urban lawns for the lower 48 states is 163 800 ± 35 850 km^2, which exceeds by three times the area of other irrigated crops (*28*). Given that these lawns can be intensively managed, there is concern that they could have important effects on regional CH_4 budgets.

In addition to conversion of native ecosystems, urban and suburban land use change has more subtle effects on ecosystem processes via increases in atmospheric N deposition and CO_2 concentrations (*24*). These increases can have strong effects on CH_4 uptake in both natural and human-altered components of urban and suburban landscapes (*18, 29*). All urban effects need to be evaluated in the context of other greenhouse forcings associated with land use change, for example, soil nitrous oxide (N_2O) and CO_2 fluxes, fossil fuel consumption.

In the Baltimore Ecosystem Study (BES, http://beslter.org), one of two urban components of the U.S. National Science Foundation's Long-term Ecological Research (LTER) network, we have established a series of long-term study plots to evaluate different components of the urban landscape (*30, 31*). Eight forest plots established in 1998 in urban and rural parks allow for evaluation of the effects of atmospheric changes associated with urbanization on intact natural ecosystems (*32*). Four lawn plots established from 1999 to 2001 allow for comparison of native forests with lawns, the most common cover type in urban, suburban and exurban areas (*28*). Data on N and C cycling and N_2O fluxes have been presented elsewhere (*30, 31*). Here we present data on CH_4 fluxes from these plots over a period of significant climatic variability (2001−2005). Our objectives were to (1) evaluate the effects of urban atmospheric and land use change on CH_4 uptake and (2) to evaluate the importance of these changes relative to other greenhouse forcings in the urban

* Corresponding author phone: 845-677-7600, ext. 128; fax: 845-677-5976, e-mail: groffmanp@caryinstitute.org.

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TABLE 1. Management/Characteristics and Soil Classification in BES Long-Term Study Plots in Baltimore, MD[a]

site	land cover	management /characteristics	soil classification
McDonogh 1	lawn	mowing three or four times a year, horse manure applied occasionally	Chester (fine-loamy, mixed, mesic typic Hapludult)
McDonogh 2	lawn	mowing once or twice a year, no fertilizer	Glenelg (fine-loamy, paramicaceous, mesic typic Hapludult)
UMBC 1	lawn	fertilizer (97 kg N ha^{-1}), Herbicide (2,4-D, prodiamine, MCPP, dicamba), biweekly mowing	Joppa (loamy-skeletal, siliceous, semiactive, mesic typic Hapludult)
UMBC 2	lawn	fertilizer (195 kg N ha^{-1}), Herbicide (2,4-D, dicamba, MCPP), weekly mowing	Brandywine (sandy-skeletal, mixed mesic typic Dystrudept)
Leakin 1	forest	high inherent fertility, undisturbed vegetation	Legore (fine-loamy, mixed, mesic, ultic Hapludalf)
Leakin 2	forest	low inherent fertility, undisturbed vegetation	Occaquon (loamy-skeletal, mixed, subactive typic Dystrudept)
Hillsdale 1	forest	high inherent fertility, undisturbed vegetation	Jackland (fine, smecitic, mesic Typic Hapluaf)
Hillsdale 2	forest	high inherent fertility, evidence of soil disturbance, extensive exotic species	Jackland (fine, smecitic, mesic typic Hapluaf)
Oregon Ridge Topslope 1	forest	low inherent fertility, undisturbed vegetation, top slope position	Glenelg (fine-loamy, paramicaceous, mesic typic Hapludult)
Oregon Ridge Topslope 2	forest	low inherent fertility, undisturbed vegetation, top slope position	Glenelg (fine-loamy, paramicaceous, mesic typic Hapludult)
Oregon ridge Midslope 1	forest	low inherent fertility, undisturbed vegetation, mid slope position	Glenelg (fine-loamy, paramicaceous, mesic typic Hapludult)
Oregon ridge Midslope 2	forest	low inherent fertility, undisturbed vegetation, mid slope position	Manor (coarse-loamy, paramicaceous, semiactive, mesic typic Dystrochrept)

[a] The Hillsdale and Leakin sites are "urban forests" and the Oregon Ridge sites are "rural forests." Forest plots are described in more detail in Groffman et al. (2006).

TABLE 2. Soil Organic Matter, pH, Bulk Density, and Extractable NH_4^+ and NO_3^- Content in BES Long-Term Study Plots in Baltimore, MD, in Summer 2000[a]

site	land cover	organic matter (%)	pH	bulk density (g cm^{-3})	NH_4^+ (mg N kg^{-1})	NO_3^- (mg N kg^{-1})
McDonogh 1	lawn	6.4	5.6	1.3	0.7	2.5
McDonogh 2	lawn	5.5	5.4	1.4	0.3	3.1
UMBC 1	lawn	1.8	4.1	ND	0.7	0.1
UMBC 2	lawn	3.7	5.0	ND	0.5	1.9
Leakin 1	forest	4.1	4.1	1.2	0.7	0.2
Leakin 2	forest	3.5	3.5	1.3	4.7	<0.1
Hillsdale 1	forest	5.9	3.5	1.0	2.1	0.1
Hillsdale 2	forest	4.8	4.0	ND	0.6	0.8
Oregon Ridge Topslope 1	forest	7.1	3.9	0.8	5.5	<0.1
Oregon Ridge Topslope 2	forest	5.3	3.9	1.1	2.7	<0.1
Oregon Ridge Midslope 1	forest	6.2	4.0	1.0	1.3	<0.1
Oregon Ridge Midslope 2	forest	5.9	4.0	1.2	1.7	<0.1

[a] The Hillsdale and Leakin sites are "urban forests", and the Oregon Ridge sites are "rural forests." Data extracted from Groffman et al. (in press).

landscape. We hypothesized that CH_4 uptake would be markedly lower in lawns and urban forests than in rural forests, and that this change would be a significant greenhouse forcing in the region (the State of Maryland).

Methods

The BES network of long-term study plots consists of eight forested and four lawn plots (Tables 1 and 2). The forest plots have been extensively described elsewhere (*30*) and were established in 1998 in remnant forests in public parks. Sampling at one of the forest plots (Hillsdale 2) was discontinued in 2005 because of continuing vandalism. Vegetation on the forest plots is dominated by tulip poplar (*Liriodendron tulipifera*) and oaks, primarily chestnut (*Quercus prinus*), scarlet (*Quercus coccinea*), and white (*Quercus alba*). Four of the forest sites, those in Leakin and Oregon Ridge parks are located in extensive forest tracts (>100 ha) and are more than 100 m from roads or houses. The plots in Hillsdale Park are in a smaller tract, less than 100 m from a high density urban neighborhood. The plots are 1600 m^2, except for the plots in Hillsdale Park, which are 900 m^2.

The lawn plots (100 m^2) were established in 2001 and include unfertilized, infrequently mowed plots, as well as plots with high inputs of fertilizer and herbicides and frequent mowing and thus represent the wide range of conditions found in typical urban and suburban lawns. Dominant grasses on the lawns include Kentucky bluegrass (*Poa pratensis*), tall fescue (*Festuca arundinacea* spp.), fine fescue (*Festuca* spp.), and white clover (*Trifolium repens*). The plots are "institutional lawns" on the campuses of a secondary school and a University, were previously in agricultural land use, and have been managed in the same way for more than 10 years. There was no evidence of alteration of the soil profile by either addition or subtraction of material at any of our lawn sites. The plots receive minimal foot traffic.

Soil/atmosphere fluxes of CH_4 were measured using an in situ chamber design. The lawn plots had chambers identical to those used by Bowden et al. (*33*). These (three

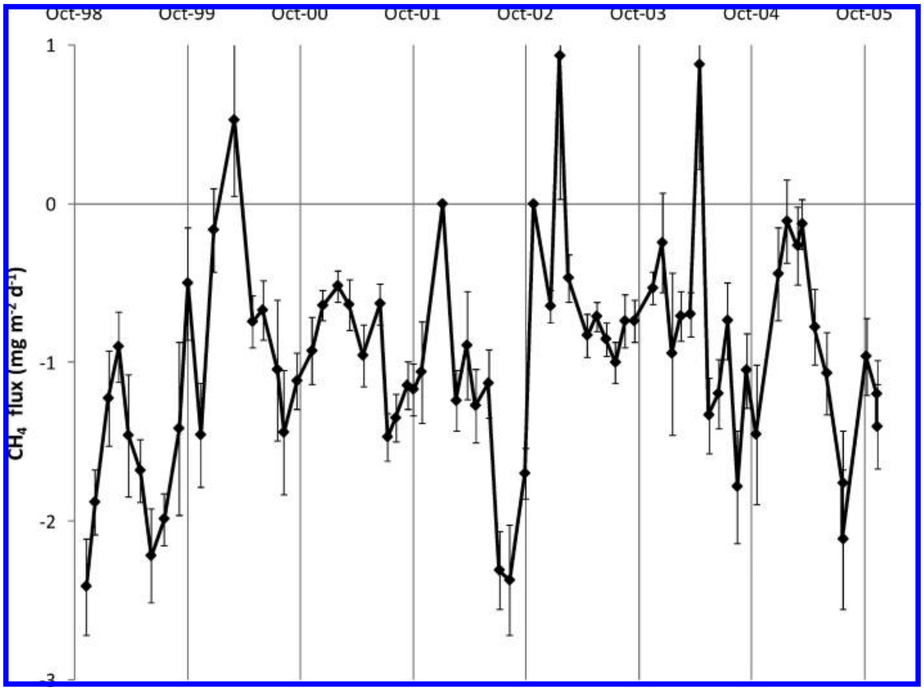

FIGURE 1. Mean (with standard error) soil/atmosphere fluxes of CH_4 over all chambers on all plots sampled approximately monthly from November 1998 through December 2005. Up to 8 forest and 4 lawn plots were sampled on each date, with three replicate chambers per plot.

per plot, at least 5 m apart and at least 5 m from the plot boundary) consisted of 28.7-cm diameter (ID) by 4.0-cm high polyvinyl chloride (PVC) cylinders which were placed on permanently installed PVC base rings immediately prior to measurement. The forest plots had the chambers described by Goldman et al. (*18*), constructed from 16.5 cm wide by 20 cm long pieces of PVC pipe fitted with a septum and an airtight well cap. These chambers were placed 4 cm into the soil and had a total volume of 2 L. At 0, 10, 20, and 30 min following placement of the chamber on the base or installation of the well cap, 9-mL gas samples were collected from gas sampling ports in the center of the chamber top by syringe. Samples were transferred to evacuated glass vials which were stored at room temperature prior to analysis by gas chromatography with flame ionization detection. Fluxes were calculated from the linear rate of change in gas concentration, the chamber internal volume, and soil surface area (*34*).

Soil moisture was measured whenever CH_4 fluxes were measured. Time domain reflectometry waveguide probes from SoilMoisture Equipment Corporation were installed horizontally into the soil at 10 cm depth. A SoilMoisture Trase System I (Model 6050 $\times$ 1, Version 2000 Software), was used to measure soil moisture. Soil (0−10 cm depth) texture was measured on the forest sites using the hydrometer method (*35*).

Given concerns about unequal sample sizes and non-normally distributed data, differences among land-use types (four replicate sites for lawn, urban forest, rural forest) were evaluated using nonparametric analysis of variance (Wilcoxon scores, Kruskal−Wallis test) using the NPAR1WAY procedure in the Statistical Analysis System (*36*). Differences among site means and years were evaluated with one-way analysis of variance with a Fisher's protected least significant difference test to determine specific differences among sites and years.

Results

Mean CH_4 fluxes over all sites were dominantly negative, indicating uptake from the atmosphere (Figure 1). The highest consumption rates occurred during summers and the lowest rates were observed during winter (Figure 1).

Because lawn plots were not established until 2001 and sampling ended at one of the forest sites in 2005, mean site fluxes were compared over all sampling dates between June 2001 and December 2004 (Figure 2). Rural forest sites had the highest consumption (1.68 mg m^{-2} day^{-1}), followed by the urban forest sites (0.23 mg m^{-2} day^{-1}) and then the lawns, which had negligible fluxes (Table 2, Figure 2, all differences $p < 0.05$).

Because sampling was discontinued at one of the urban forest sites, temporal comparisons between lawn and forest sites are based on data from the seven remaining forest sites versus the four lawn sites, and run from June 2001 through December 2005. Fluxes were higher ($p < 0.0001$) in forests than lawns every year (Figure 3). In an analysis over all forest and lawn plots, uptake was highest ($p < 0.05$) in 2002, a very dry year (68% of average annual precipitation since 1963 at the NOAA weather station at Baltimore Washington International airport, < 50 km from our sites), and lowest ($p < 0.05$) in 2003, a very wet year (148% of average precipitation).

There were significant negative relationships between CH_4 uptake and soil moisture in the forest (rural and urban combined) plots ($r = 0.44$, $P < 0.0001$) but not in the lawn plots ($r = 0.09$, $p < 0.25$) (Figure 4). The relationship was similar in the rural (Figure 4a, $r = 0.26$, $p < 0.0001$) and urban forest sites (Figure 4b, $r = 0.21$, $p < 0.0033$). However, there was a wider range of moisture content in the urban forest plots, and over all dates, moisture was higher ($p < 0.0001$) in the urban forest plots than the rural forest plots (Table 3). CH_4 fluxes were higher in rural than urban forest sites, even when sites were compared within controlled ranges of soil moisture (0 − 10% and 10 − 20%) (Table 3). There were no significant differences in % sand (47 $\pm$ 7% in rural versus 39 $\pm$ 16% in urban) or % clay (14 $\pm$ 4% in rural, 13 $\pm$ 2% in urban) in the top 10 cm of soil between rural and urban forest sites.

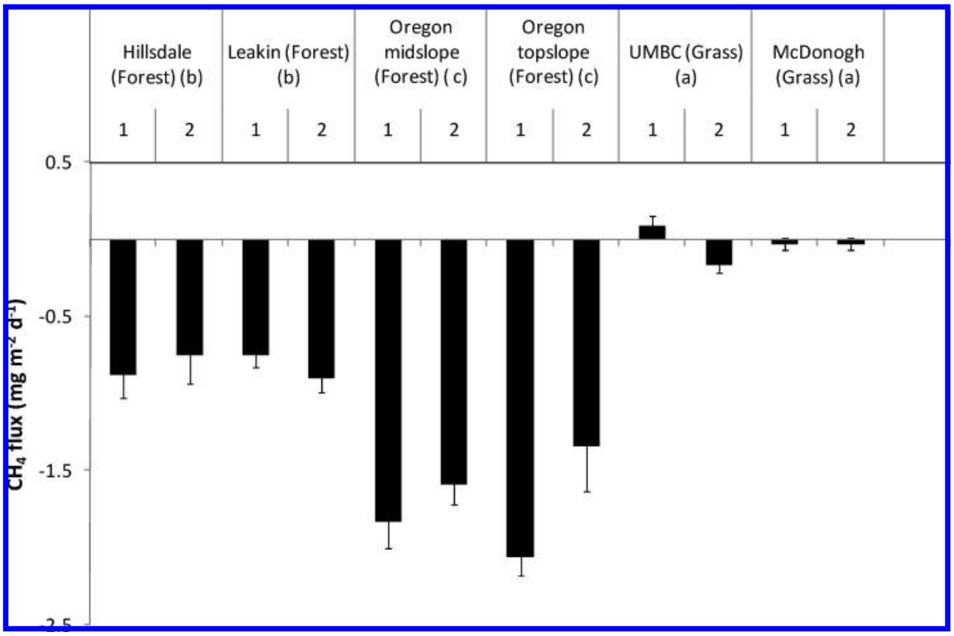

FIGURE 2. Site mean soil:atmosphere fluxes of CH_4 in 8 forest and 4 lawn plots in the Baltimore metropolitan area. The Hillsdale and Leakin sites are "urban forests" and the Oregon Ridge sites are "rural forests." Values are means of all fluxes measured in 3 replicate chambers per plot from June 2001 through May 2004. Sites followed by different letters are significantly different at p < 0.05, i.e., sites marked a are different than sites marked b and c.

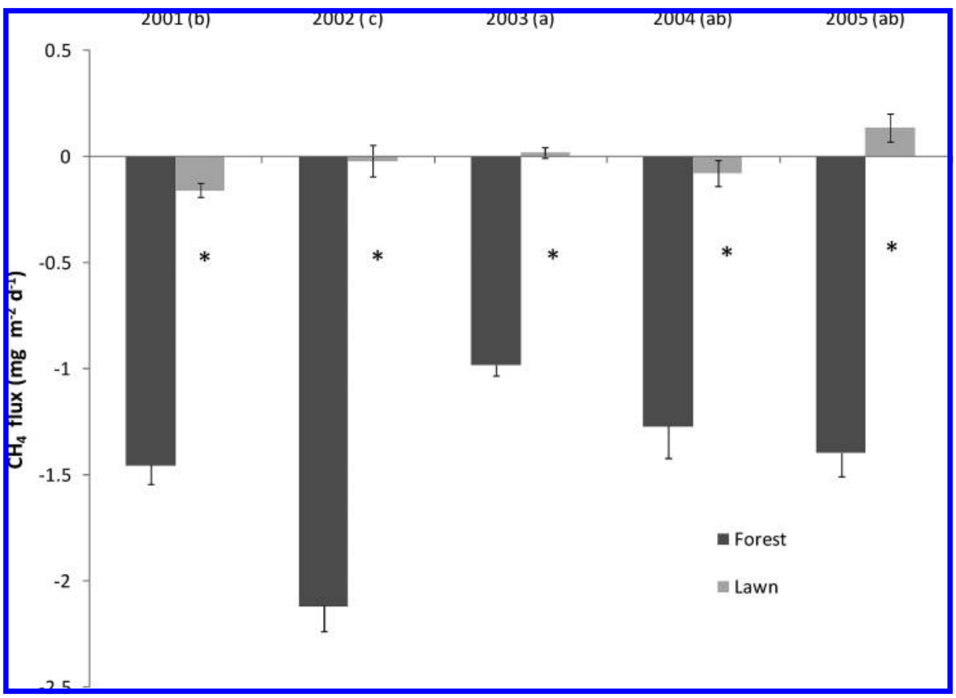

FIGURE 3. Soil/atmosphere fluxes of CH_4 from forest and lawn plots in the Baltimore metropolitan area from June 2001 to December 2005. Values are means from 3 replicate chambers in 7 undisturbed forest and 4 grass plots. *Indicates significant difference between forest and grass at p < 0.05. Years followed by different letters are significantly different at p < 0.05, e.g. 2002 is lower than 2001, which is lower than 2003.

Discussion

Why Was There so Little CH_4 Uptake in Urban Lawns? The most marked result that we observed was the low rates of CH_4 uptake in urban lawns compared to forests. Given that many studies, including several in the northeastern U.S., have shown that N additions have an inhibitory effect on CH_4 uptake (*7, 18, 37*), we assume that differences in N dynamics between forests and lawns play a large role in this result. However, relationships between N additions and cycling are not straightforward in either this or previous studies (*38*). Our lawns represented a wide range of N management; some sites received no fertilizer, while others were heavily fertilized (200 kg N ha^{-1} year^{-1}), yet all lawn sites had very low CH_4 fluxes. It is also important to note that the "lawn effect" on CH_4 uptake that we observed is much greater than results from previous studies that report inhibitory effects of N additions to forest and grassland soils generally in the range of 25−50%, not the near complete inhibition that we observed here (*7, 8, 37, 39, 40*). In the study most similar to ours, Kaye et al. (*19*) found only a 50% reduction in uptake in urban lawn, corn and wheat-fallow soils compared to native grasslands in Colorado. The extreme

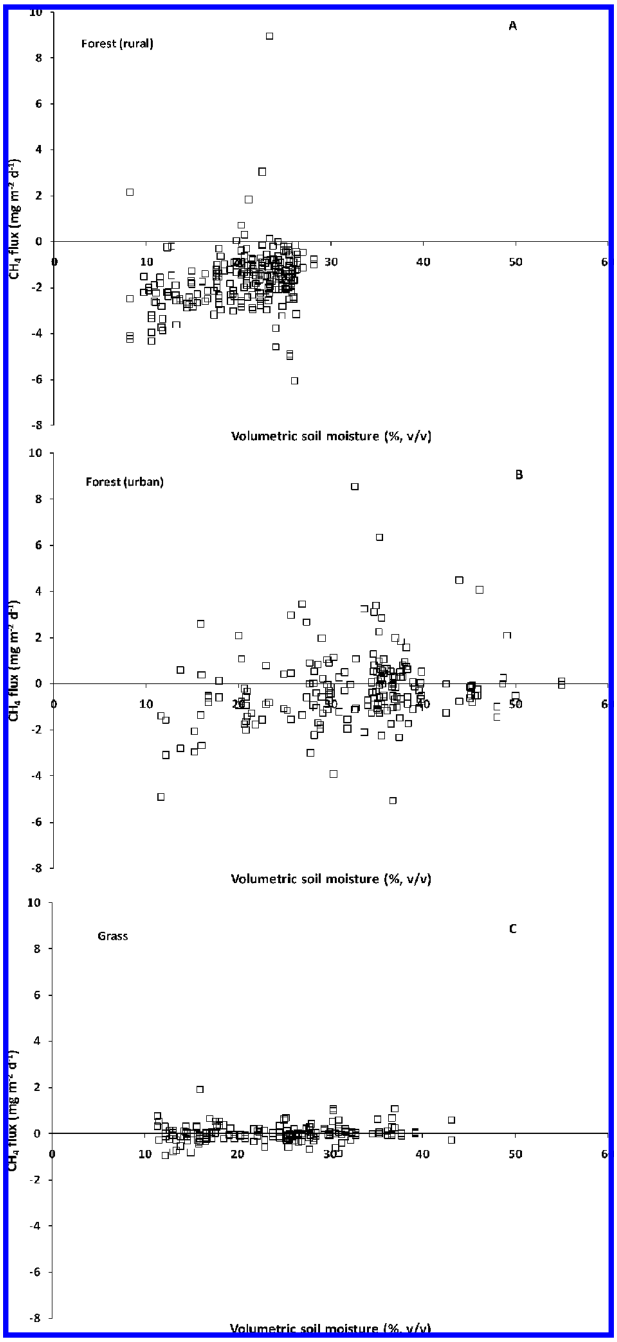

FIGURE 4. CH_4 flux versus soil moisture in rural forest (A), urban forest (B) and urban grassland (C) sites in the Baltimore metropolitan area from 1998 to 2005.

effects that we observed, and the lack of systematic relationships between N inputs and inhibition, suggest that there are characteristics of urban lawns other than N dynamics that have important effects on CH_4 uptake.

In addition to N, CH_4 uptake is strongly affected by soil moisture, which has a strong effect on diffusion, which is essential for the movement of CH_4 from the atmosphere to the microorganisms that oxidize it in the soil (*15, 41*). While there were no consistent differences in soil moisture between our forest and lawn sites (*31*), the nature of the soil/atmosphere interface differs markedly between forests and lawns (*42*). If these differences inhibit exchange of gases between the soil and the atmosphere, they could reduce the flow of CH_4 from the atmosphere to oxidizing microbes in the soil. Such an effect has been observed in comparisons of spruce and beech forests, where the dense structure of the surface soil layer in spruce forests inhibits diffusion of CH_4 from the atmosphere to the soil (*43*).

TABLE 3. CH_4 Flux in Rural and Urban Forests Compared over the Full Range of Soil Moisture Contents Observed As Well within Two Specific Ranges of Soil Moisture (10−20% and 20−30%)[a]

	rural forests		urban forests
all moisture contents			
CH_4 flux (mg m^{-2} d^{-1})	−1.68 (0.09)	[b]	−0.23 (0.12)
soil moisture (%, v/v)	20.4 (0.3)	[b]	32.3 (0.7)
Number of observations	233		190
10−20% moisture			
CH_4 flux (mg m^{-2} d^{-1})	−2.22 (1.0)	[b]	−1.24 (0.40)
soil moisture (%, v/v)	14.8 (0.3)		15.1 (0.5)
number of observations	75		18
20 − 30% moisture			
CH_4 flux (mg m^{-2} d^{-1})	−1.40 (0.11)	[b]	−0.42 (0.19)
soil moisture (%, v/v)	23.5 (0.2)	[b]	26.0 (0.5)
number of observations	154		56

[a] Values are mean (standard error). [b] Indicates statistically significant difference between rural and urban forests at $p < 0.01$.

Several other studies have observed surprising differences in ecosystem capacity for CH_4 uptake and have suggested that differences in the populations of CH_4 oxidizing organisms may play an important role in these differences. Singh et al. (*44*) found lower oxidation in pastures (reduced up to 70%) versus pine forests and shrubland in New Zealand and suggested that differences in methanotroph populations in the pastures were responsible for the differences. Menyailo et al. (*14*) found that afforestation of grasslands resulted in a marked decline in CH_4 uptake because of declines in methanotroph populations. In contrast, Ambus and Robertson (*45*) found no difference in CH_4 uptake between unmanaged forest and grassland communities on abandoned agricultural areas in Michigan. There is a clear need for research on the ecosystem-scale factors that influence the development and maintenance of methanotroph populations.

Is There an Urban Atmospheric Effect on CH_4 Uptake in Urban Forests? While the CH_4 uptake rates observed in our rural forests were similar to those measured at forested sites in the northeastern U.S. (*6*), our urban forest sites had significantly lower uptake. Given the many studies discussed above showing that N additions have an inhibitory effect on CH_4 uptake, one possibility is that high rates of atmospheric N deposition in the urban core of Baltimore are responsible for the low rates of uptake that we observed in our urban forest sites. While we have no data on atmospheric deposition at our sites, there is great potential for atmospheric deposition to be elevated in urban areas (*46−49*). It is also important to note that N additions do not always lead to reductions in CH_4 uptake by forest soils (*45, 50, 51*). Further studies are needed to determine if atmospheric N deposition is playing a role inhibiting CH_4 uptake in urban forests.

In addition to atmospheric N deposition, CH_4 uptake in the urban forest sites may also have been reduced by the high levels of atmospheric CO_2 that have been observed in the urban core of Baltimore (*52*). Phillips et al. (*53*) found 16−30% inhibition of CH_4 uptake in forest plots continuously enriched with CO_2 at 200 μL L^{-1} above ambient. They did not observe differences in soil moisture in enriched plots, suggesting that changes in CH_4 uptake were the result of changes in the composition or size of the CH_4 oxidizing community. These changes may have been induced by competition for oxygen under elevated CO_2, which may have negatively affected the size, composition and/or activity of the CH_4 oxidizing community.

Our comparison of urban and rural forests is complicated by differences in soil moisture and other factors that affect diffusion of CH_4 from the atmosphere to the soil. The urban forest soils were wetter than the rural forest soils, which likely accounts for much of the difference that we observed. However, uptake was still lower in urban forest soils when the comparison was controlled to similar soil moisture levels, suggesting that some other factor contributed to the "urban effect." In addition to soil moisture, diffusion is controlled by soil texture, structure and horizonation (*43, 54, 55*). There are definite hydrologic differences among our sites, e.g., zero tension lysimeters collect water with different efficiency in our rural and urban forest sites, suggesting physical differences in the soil profile (*30, 31*). Physical differences in the soil profiles of our urban and rural sites are also suggested by the fact that soil moisture was consistently higher in the urban sites despite similar soil texture. So we cannot rule out differences in diffusion other than those caused by soil moisture affecting our uptake rates. Further research is needed to determine just what factor or combination of factors (atmospheric N, atmospheric CO_2, diffusion) is responsible for the differences in CH_4 uptake between our urban and rural forest sites.

Global Warming Impact of Changes in CH_4 Flux Associated with Land Use Change. Although conversion of native forests to urban lawns has dramatic effects on CH_4 uptake, these effects do not appear to be significant to statewide greenhouse gas forcing. We used the methodology presented by Robertson et al. (*56*) to assess the global warming impact of changes in CH_4 flux associated with land use change. The mean difference in flux between rural forests and urban lawns (1.68 mg m^{-2} day^{-1}) yields a global warming potential of 14.7 g C m^{-2} $year^{-1}$ for urban lawns. If we assume that 10% of the land area of Maryland is covered by turfgrass (*28*), then the CH_4 impacts of conversion of native forest to turf produce the global warming equivalent of 37.2×10^3 metric tons of C per year.

Given per capita C emissions for the U.S. of 5.32 t (http://cdiac.ornl.gov/trends/emis/top2005.cap), the 37.2×10^3 tons of C associated with conversion of native forest to urban lawns is equivalent to the annual emissions of 6997 people. Maryland has a total population of approximately 5.6 million.

Cattle are a significant source of CH_4, emitting approximately 100 kg CH_4 per capita per year (http://www.epa.gov/rlep/faq.html). The 37.2×10^3 tons of C associated with conversion of native forest to urban lawns are thus equivalent to the emissions from 15 516 cattle. There were 220 000 cattle and calves in Maryland in 2007 (http://www.msa.md.gov).

The 37.2×10^3 metric tons of C associated with urban lawns are also equivalent to the C content of 15 639 495 gallons of gasoline or the annual C emissions of 32 582 cars (assuming 25 miles per gallon and 12 000 miles per year). Approximately 400 000 new cars were purchased in Maryland each year between 2003 and 2007 (http://www.marylandmva.com/).

This assessment of the global warming impact of changes in CH_4 flux associated with land-use change is based on conversion of rural forest to lawn. Much of the current land-use change in Maryland involves conversion of agricultural lands, which have a lower CH_4 uptake capacity than forests (*16*), to lawns (*23*). Our calculations therefore represent the upper limit of the contemporary (as opposed to long-term historic) effect of urban and suburban lawns on statewide CH_4 uptake greenhouse gas forcing.

It is also important to note that changes in CH_4 uptake are only one component of the potential greenhouse gas forcing associated with urban and suburban lawns. A more complete accounting would include assessment of changes in carbon stored in vegetation and soil, and fossil fuel used in lawn maintenance activities. While the changes in CH_4 uptake do not appear to be significant to statewide greenhouse gas forcing, these other changes may be more significant.

Acknowledgments

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