Methane flux in non-wetland soils in response to nitrogen addition: a meta-analysis

E. L. Aronson[1] and B. R. Helliker

433 South University Avenue, Leidy Labs, University of Pennsylvania, Philadelphia, Pennsylvania 19104 USA

Abstract. The controls on methane (CH_4) flux into and out of soils are not well understood. Environmental variables including temperature, precipitation, and nitrogen (N) status can have strong effects on the magnitude and direction (e.g., uptake vs. release) of CH_4 flux. To better understand the interactions between CH_4-cycling microorganisms and N in the non-wetland soil system, a meta-analysis was performed on published literature comparing CH_4 flux in N amended and matched control plots. An appropriate study index was developed for this purpose. It was found that smaller amounts of N tended to stimulate CH_4 uptake while larger amounts tended to inhibit uptake by the soil. When all other variables were accounted for, the switch occurred at 100 kg N·ha^{-1}·yr^{-1}. Managed land and land with a longer duration of fertilization showed greater inhibition of CH_4 uptake with added N. These results support the hypotheses that large amounts of available N can inhibit methanotrophy, but also that methanotrophs in upland soils can be N limited in their consumption of CH_4 from the atmosphere. There were interactions between other variables and N addition on the CH_4 flux response: lower temperature and, to a lesser extent, higher precipitation magnified the inhibition of CH_4 uptake due to N addition. Several mechanisms that may cause these trends are discussed, but none could be conclusively supported with this approach. Further controlled and in situ study should be undertaken to isolate the correct mechanism(s) responsible and to model upland CH_4 flux.

Key words: CH_4; meta-analysis; methane; methanogen; methanotroph; N addition; N deposition; soils.

Introduction

Methane (CH_4) contributes up to 30% to the total net anthropogenic radiative forcing of the atmosphere (Solomon et al. 2007), yet little is known about the year-to-year controls on CH_4 variation (Bousquet et al. 2006). In most of the last 150 years, atmospheric CH_4 has increased monotonically. The CH_4 concentration in the atmosphere became erratic and did not increase overall from 1999 until 2007, and then began increasing again (Rigby et al. 2008). Several explanations have been proposed for the recent vagaries in the growth of atmospheric CH_4 including variations in wetland sources, the atmospheric concentration of OH^- radicals (Rigby et al. 2008) and the ocean-air chlorine sink (Schaefer and Whiticar 2008). The wide range of potential explanations for atmospheric CH_4 growth trends indicates a basic lack of understanding of the interplay between biotic and abiotic controls on CH_4 cycling. There exists, however, a growing body of research into soil CH_4 flux responses to environmental conditions that can be used to augment our insight into this important gas from the bottom up. Of the environmental factors that have the greatest impact on

CH_4 flux, which include temperature, precipitation, nitrogen (N) status and oxygen penetration, inorganic N has been experimentally manipulated the most often and with the most varied results.

Of all the uncertainties in CH_4 sources and sinks, the biotic sink is the most variable, especially in relation to human activities. The most common figure for gross annual CH_4 oxidation in soil is ~30 Tg CH_4 (Solomon et al. 2007). This figure is often presented without a specific citation (as in Lelieveld et al. 1998) and has been based on top-down modeling approaches that assign the difference between the total sources, atmospheric concentration, and destruction to the "terrestrial sink." Only recently have attempts been made to model from local observations, and these have left large uncertainties (Zhuang et al. 2004, Dutaur and Verchot 2007).

Soil exchange of CH_4 with the atmosphere is regulated by two groups of microorganisms, generally referred to as methanogens and methanotrophs. The disparate environmental requirements of these two groups, particularly with regards to oxygen, water, and nutrient levels, determine the CH_4 flux of a given soil. Methanogens, active in anaerobic conditions, produce CH_4 as a byproduct of metabolism and are the main biological source of CH_4 across systems. Methanotrophic bacteria are active in aerobic conditions, derive energy and carbon from the oxidation of

CH$_4$, and are its only known biotic sink (Hanson and Hanson 1996). High affinity methanotrophic bacteria are considered to be responsible for consuming CH$_4$ at atmospheric levels in well-drained soils. These have never been cultivated (Kolb 2009). There is a growing body of information from the last 20 years on these microorganisms that is based on biomarker (Holmes et al. 1999) and stable isotope probing (Dumont and Murrell 2005) approaches, which have linked CH$_4$ oxidation in forest soil with the molecular signatures of uncultured methanotrophs. As these have yet to be cultured, they are referred to by taxonomic cluster types, including upland soil cluster α (USCα), dominant in acidic soils, and USCγ, associated with some members of the Methylocystaceae in pH-neutral soils (Kolb 2009).

The effects of N on soil CH$_4$ flux are understood less than those of other environmental variables, such as moisture and temperature. The overall N input from deposition and fertilizer is projected to double from 1990 levels by the year 2050 (Kroeze and Seitzinger 1998). The current average available N deposition on the Earth's terrestrial surface is estimated to be greater than 64 Tg N/yr (Galloway et al. 2004), although it is not evenly distributed. Fertilization rates can be two orders of magnitude higher in cropland, and total greater than 170 Tg N/yr (Galloway et al. 2004). Cropland and pasture consume less CH$_4$ than natural forest and grassland (Ojima et al. 1993, Willison et al. 1995). This is at least partially due to the strong evolutionary links between the genetics behind enzymes responsible for CH$_4$ and ammonia oxidation, which allow methanotrophs and ammonia oxidizers to switch substrates (Dunfield and Knowles 1995). This mechanism is believed to be responsible for the inhibition of CH$_4$ uptake by soil exposed to high concentrations of available N (Hanson and Hanson 1996). However, smaller concentrations of available N relative to available CH$_4$ can result in N limitation of CH$_4$ uptake in wetland systems (Bodelier and Laanbroek 2004).

The focus of this meta-analysis is to determine (1) the critical variables that control terrestrial CH$_4$ fluxes and (2) the impact of soil N addition, and its interaction with other variables, on these fluxes. There is a large innate variability in CH$_4$ flux within and across sites, but this variability may be governed by large-scale abiotic factors. This meta-analysis has the potential to make site-specific results useful for modeling at regional and global scales (Rustad et al. 2001); and may be used to predict the impact that increased N deposition from industry, N fertilization, and N runoff will have on various ecosystems. This meta-analysis ties the magnitude of the experimental N influx to the resultant CH$_4$ flux across ecosystems. These results are timely, as patterns of temperature and rainfall are shifting and N deposition and fertilization increasing.

METHODS

Data sources

Methane flux data were extracted from published studies that contained matched N addition and control treatments (listed in Appendix A). We limited the inclusion of studies to systems that are traditionally thought to take up more CH$_4$ than they release: non-wetland, terrestrial ecosystems. Analysis was limited to the measurement of aerobic oxidation of CH$_4$ under ambient CH$_4$ concentrations; uptake by anaerobic oxidation or under elevated [CH$_4$] was not considered. All included studies used intact soil, mostly in situ with the exception of Kruse and Iverson (1995) and Willison et al. (1995), which used soil cores exposed to atmospheric CH$_4$ concentrations soon after removal from the field. All studies used static (mostly vented) chambers (Hutchinson and Mosier 1981). All the original data are extracted from figures and tables in the published papers. The studies were located using review papers (Le Mer and Roger 2001, Dutaur and Verchot 2007) and ISI Web of Knowledge using search terms: "methane" and "uptake," "oxidation," "flux," or "consumption" and "fertilization" and "nitrogen addition" or "nitrogen deposition." The resultant database from 33 papers consisted of 79 entries, each containing a single added N vs. control comparison. There were multiple comparisons from many studies due to different levels of N treatment or factorial designs. The annual CH$_4$ uptake averages presented in primary or secondary literature were used when applicable, while averages were calculated based on figures if no yearly average was provided. All flux measurements were standardized to a flux density of CH$_4$ in mg·m^{-2}·yr^{-1}.

Ancillary information from each data source included latitude, average daily temperature and precipitation during study, duration of study, biome, ecosystem type, soil management status, season studied, form of N fertilizer used, amount of N used, and collection method and intervals. It was also recorded whether the plots had been fertilized long term or only for the duration of the study and, separately, whether fertilizer had only been applied before the study or also during the study. The forms of fertilizer used were ammonium, nitrate, urea, glycine, nitric acid, and NPK (unknown N species). As glycine and nitric acid were used as N sources in only one study each (Christensen et al. 1999 and Bradford et al. 2001, respectively), the more common N species that result from the first steps of decomposition of these molecules (ammonium and nitrate, respectively) were used for analysis. The average amounts and standard deviation of each type of fertilizer used are summarized in Appendix B.

In order to allow the better methane flux estimates to influence the analyses more than those that may be less accurate, a weighting scheme was used for all analyses unless otherwise noted. This scheme weighted the data by the level of representation of the ecosystem it studied

in terms of the duration of study in years, seasons studied (i.e., fractions of years), and frequency of collection. Each season constituted 0.25, the collection period was extrapolated to the number of flux measurements on different days in a year, and these were each multiplied together with the number of years of the study. Therefore a study of one year that only took eight flux measurements across the summer (i.e., at a rate of 32 measurements per year) would have a weight of $1 \times 0.25 \times 32$, yielding 8, while a four-year, year-round study with 32 measurements annually would be weighted $4 \times 1 \times 32$, yielding a weight of 128, appropriately larger relative to the accuracy of representation of that ecosystem.

If the temperature and precipitation during the study were not presented in the publication, the authors were contacted for this information. When the authors were unable to provide this information, the NOAA Climate Data Online system was searched for the actual period of CH_4 flux observation, from a day before till the end of the study. For multi-year studies where only growing season flux data were taken, only the temperature during flux measurement was used with precipitation data for the entire study period, as moisture status during periods of snowfall can affect growing season water status (Gulledge et al. 1997).

Study index and analyses

The usual indices and statistics for meta-analysis either include the variance from each study or the log of the ratio of the treatment value to the control value. The studies did not always include published variances with their CH_4 flux values, so the first type of index was not applicable. When CH_4 flux values are reported, usually the uptake of CH_4 is reported with a negative value, so simple ratios lack the specificity of what change has occurred, and logarithms of negative numbers are not an option. Therefore a new study index was created for the purposes of this analysis:

$$T_i = \pm \frac{|N_i - C_i|}{|C_i| + |N_i|} \qquad (1)$$

where C_i and N_i indicate an individual, paired, average measurement of CH_4 flux of control (C_i) and N treatment (N_i) plots. In order to establish the entire absolute difference of these two quantities, one is subtracted from the other, and the resultant number is stripped of sign in the numerator. To make this quantity relative to the amount of methane the site is capable of releasing or consuming, the denominator contains the absolute value of each measurement of CH_4 flux added together. The variable T_i refers to the resulting study index, which is assigned a sign based on the direction of the difference (if any) in CH_4 flux due to N addition: a negative T_i indicates an increase in CH_4 uptake (decrease in release) due to N addition, and a positive T_i indicates a decrease in uptake (increase in release). This index is similar to the percent increase or decrease

relative to control, but is in proportion to both control and N amended uptake, instead of just the control on its own. The differences described with this statistic are therefore relative to the expected flux in that site regardless of N status. The range of T is from -1 to 1, with an index of zero indicating no difference in CH_4 flux between the control and treatment plots.

Due to great variation in soil microbial communities and their responses to environmental stimuli, as well as in pre-existing N status, it was hypothesized that the effect size, or overall difference of the effect from zero, would not be significant. Standard statistical approaches were employed in this case, in contrast to many meta-analyses (Arnqvist and Wooster 1995), because the main focus of this study was not to determine an overall effect size, but rather to cull the relative effect sizes of various factors on the response of CH_4 flux to N addition. The cumulative, annual CH_4 flux was determined as accurately as possible for each site, and could thus be used as a single data point, while differences in methodologies were represented in the weighting scheme. It was assumed that the distributions of flux responses were normal within each study site, even though data altogether were not normally distributed. It was also assumed that the variances of these responses within each study site were homogeneous. Regression, ANOVA, and other statistical tests were performed using JMP IN software (Version 8, SAS Institute, Cary, North Carolina, USA). Vote-counting, a common, non-probabilistic approach used in many literature reviews, was also performed.

RESULTS

Regression of the weighted CH_4 flux in control by treatment plots showed a tight correlation ($R^2 = 0.75$, $P < 0.0001$) with a slope of 0.85 (Fig. 1). This indicates that CH_4 uptake is larger in control plots overall. Vote-counting measures of the study index indicate an overall trend of decrease of CH_4 uptake with the addition of inorganic N. Out of the 79 comparisons, 57 showed a decrease in CH_4 uptake, 19 showed an increase, and 3 showed no change with N addition. The average of the unweighted study index was $T = 0.127$, while the weighted study index average was $T = 0.095$.

Study index responses

Stepwise multiple regression was performed on the effects of continuous variables on the study index. Overall single regressions are not presented because a multivariate correlations analysis showed that the independent variables were up to four times more highly correlated with each other than with the target variable, the study index (see Appendix B for all correlations tables). However, when these independent variables were included in a full factorial stepwise multiple regression, all factors stepped into the model at once. In the resultant standard least squares multiple regression (Table 1, model $R^2 = 0.53$), average daily
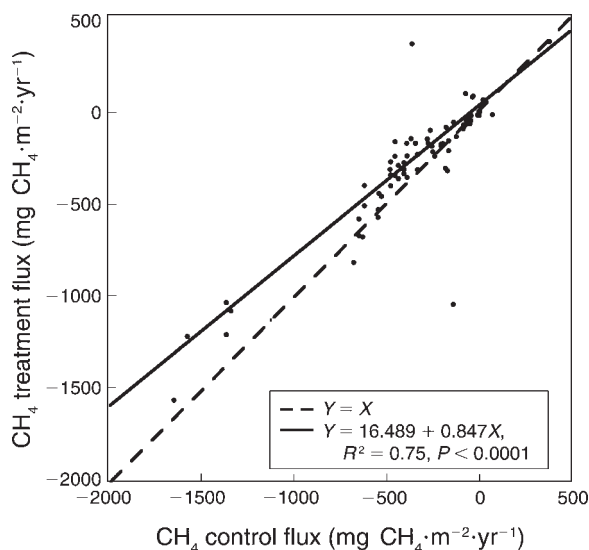
Fig. 1. The relationship between CH_4 flux in control plots and treatment plots. Negative flux values indicate atmospheric CH_4 uptake by soils. The solid line is the weighted linear fit of the points shown. The dashed line is the 1:1 line for comparison.

temperature and the interaction between average temperature and latitude both significantly affected the variance in T ($P < 0.003$ and $P < 0.0024$, respectively). The total amount of N added was also a significant factor ($P < 0.0005$), with low amounts of added N leading to a negative T value and larger amounts leading to a positive T, and the switch occurring around 100 kg $N \cdot ha^{-1} \cdot yr^{-1}$ (data not shown). Average daily precipitation was nearly significant ($P < 0.057$).

In order to more adequately investigate the impact of N addition, single regression analyses were performed on the effect of the amount of each individual N species on the study index. Nonsignificant lines of regression are not shown. The only N species that was found to significantly affect the study index was nitrate, which caused a decrease in methane uptake with greater amounts added (Fig. 2). This relationship appeared to be influenced strongly by two overlapping points (shown in gray), so the regression was performed with (gray line, $R^2 = 0.21$, $P < 0.0001$) and without (black line, $R^2 = 0.05$, $P < 0.0429$) these points.

Single regressions were also performed on the effect of total N addition on the study index when categorical variables were used to segregate the points. Although every possible combination was tried, only those of interest are presented. In the temperate biome, there was a strong ($R^2 = 0.12$, $P < 0.019$) direct relationship between the amount of nitrogen added and the study index (Fig. 3) that did not exist in the other two biomes. This relationship was also stronger ($R^2 = 0.24$, $P < 0.038$) in managed land than in land that was pristine until the study began (Fig. 4). Despite an apparent outlier, this relationship existed more strongly ($R^2 = 0.28$, $P < 0.0006$) in long-term fertilized land than land that had a shorter term of fertilization (Fig. 5).

Many categorical factors affected the variance of the study index (Fig. 6). Grassland sites responding to N addition with a more positive study index than forest sites ($P < 0.009$). Biome had no significant effect on the CH_4 uptake response ($P < 0.093$). The study index of actively managed land was more negative than of unmanaged land ($P < 0.0001$). The type of fertilizer affected on the study index ($P < 0.002$); added ammonium significantly increased uptake while added ammonium nitrate and urea decreased CH_4 uptake, and no other fertilizer type differed. Those sites that were not fertilized prior to flux measurement had a less negative study index than those that had been fertilized longer ($P < 0.005$). There was no effect of whether fertilization actually occurred during the study or only before ($P < 0.738$).

Flux responses

Stepwise multiple regression analysis was performed on the effects of fully factorial continuous variables on

TABLE 1. Effects of continuous factors on the study index.

Factors on study index	Estimate	SE	t	P
Intercept	0.158	0.566	0.280	0.781
Latitude (minutes)	0.002	0.005	0.520	0.606
Average temperature (°C)	−0.040	0.013	−3.090	0.003*
Temperature × latitude	0.002	0.000	3.160	0.002*
Average daily precipitation (mm)	0.130	0.067	1.940	0.057
Precipitation × latitude	−0.004	0.004	−1.060	0.291
Temperature × precipitation	−0.008	0.011	−0.670	0.504
Temperature × precipitation × latitude	0.000	0.000	−0.170	0.863
N added (kg $N \cdot ha^{-1} \cdot yr^{-1}$)	0.002	0.001	3.690	0.001*
Latitude × N	0.000	0.000	−0.610	0.547
Temperature × N	0.000	0.000	−0.730	0.470
Temperature × latitude × N	0.000	0.000	0.490	0.629
Precipitation × N	0.001	0.001	0.860	0.395
Precipitation × latitude × N	0.000	0.000	−0.180	0.855
Temperature × precipitation × N	0.000	0.000	−1.670	0.100
Temperature × precipitation × latitude × N	0.000	0.000	0.770	0.446

Note: For similar tables of impacts on CH_4 flux in control and nitrogen-amended plots, see Appendix B.
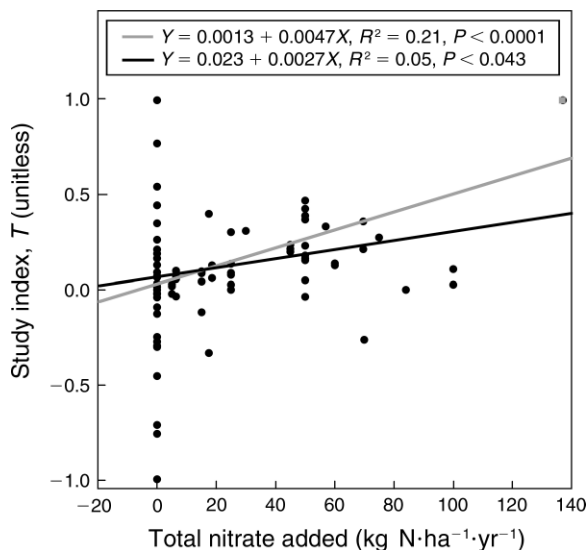* $P < 0.05$.

FIG. 2. The relationship between the amount of nitrate added to the soil and the study index, T. The gray line indicates the relationship including two possible outlier points (also in gray; points overlap). The black line is the regression of the same relationship omitting the two points in gray. Negative T values indicate increased atmospheric CH_4 consumption by soils with N addition.

FIG. 4. The relationship between the amount of N added and the study index in soils that had been managed before the study period. Negative T values indicate increased atmospheric CH_4 consumption by soils with N addition.

CH_4 flux in control and N amended plots, and all factors stepped into the model. The resultant standard least squares multiple regression tables are supplied in Appendix B (model $R^2 = 0.29$ for control and $R^2 = 0.55$ for treatment plots). Temperature significantly affected the CH_4 uptake of control plots ($P < 0.019$), as did the interactions between latitude and precipitation ($P < 0.002$), between average temperature and latitude
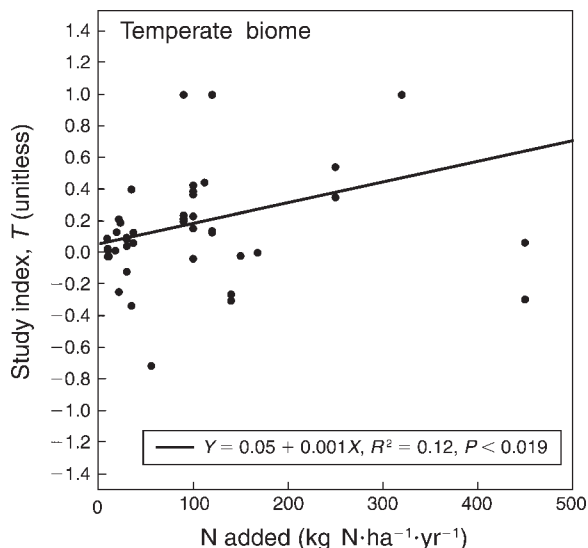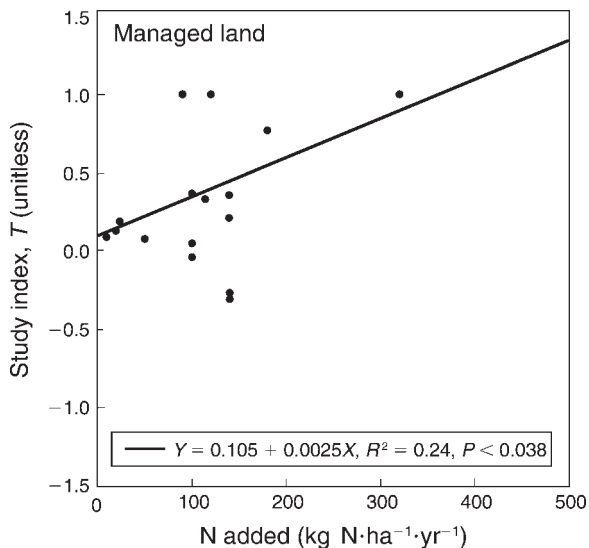
($P < 0.022$), and between average temperature and daily precipitation ($P < 0.025$). Average daily precipitation and the amount of N added significantly affected the model of N treatment CH_4 flux ($P < 0.007$ and $P < 0.005$, respectively), as did the interactions between latitude and daily precipitation ($P < 0.0001$), average temperature and daily precipitation ($P < 0.0001$), latitude, average temperature, and amount of N added ($P < 0.002$), daily precipitation and amount of N added ($P < 0.006$), latitude, daily precipitation, and amount of
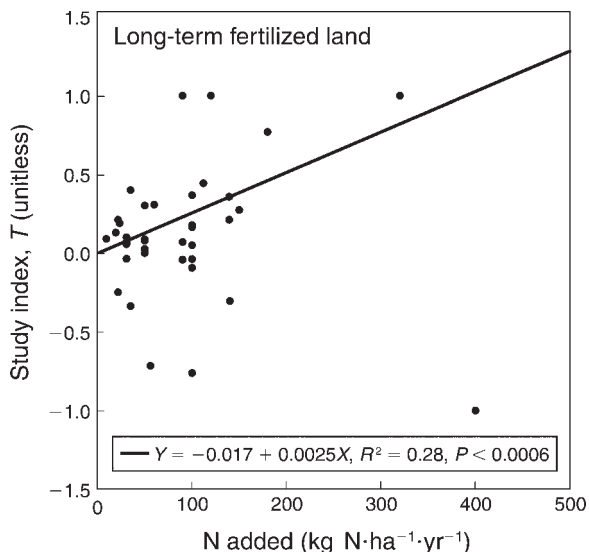

FIG. 3. The relationship between the amount of N added and the study index, T, in soils located in the temperate biome. Negative T values indicate increased atmospheric CH_4 consumption by soils with N addition.

FIG. 5. The relationship between the amount of N added and the study index in soils that had been exposed to long-term fertilization. Negative T values indicate increased atmospheric CH_4 consumption by soils with N addition.
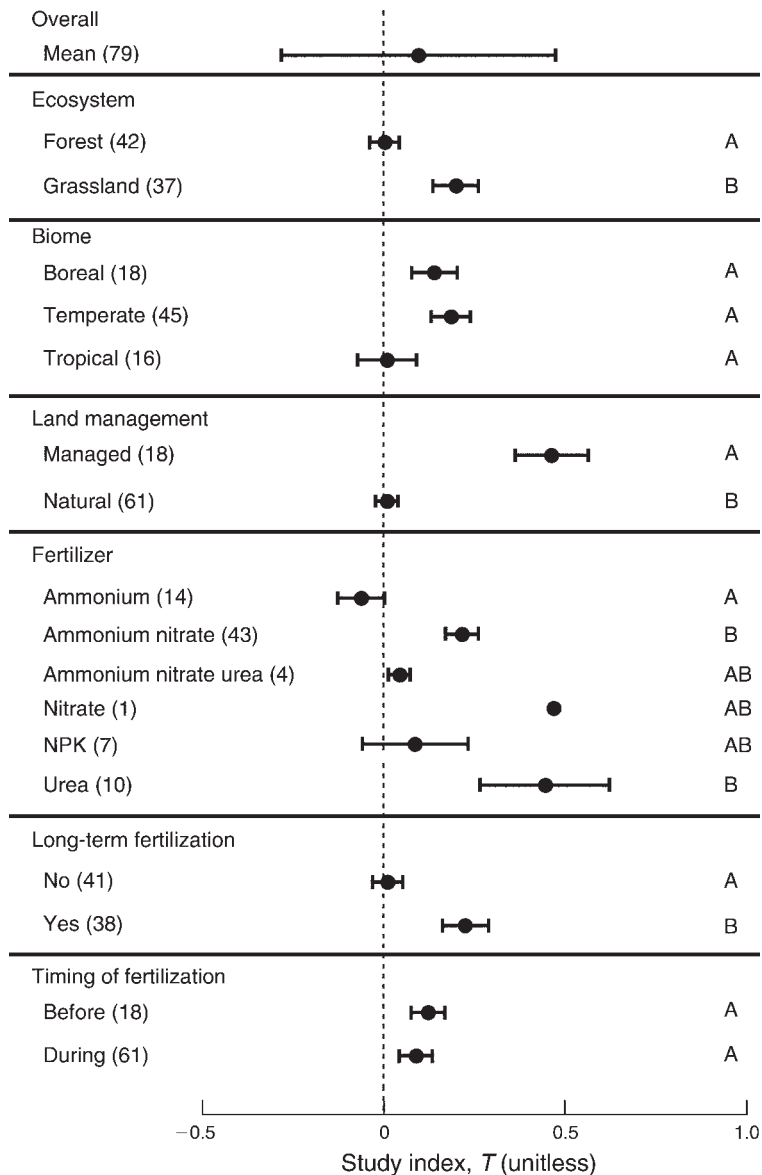
FIG. 6. Environmental and land management effects on T. Values expressed are means bounded by the standard error of the mean. Negative T values indicate increased atmospheric CH_4 consumption by soils with N addition, with sample size indicated in parentheses. Different letters indicate statistically significant differences between means (Tukey's hsd, $P < 0.05$). The dotted line is zero on the x-axis.

N added ($P < 0.023$), and average temperature, daily precipitation, and amount of N added ($P < 0.013$).

The effects of all categorical variables on the CH_4 flux are displayed in Figs. 7 and 8. In control plots (Fig. 7), forest sites were found to consume greater amounts of CH_4 than grasslands ($P < 0.014$). Temperate biome sites were found to consume more CH_4 than tropical sites, with neither significantly different from sites in the boreal biome sites ($P < 0.012$). There was no difference in CH_4 flux of control plots that were managed vs. natural. Of the categorical variables tested for N-amended plots (Fig. 8), forest sites were found to consume more CH_4 than grasslands ($P < 0.006$).

Temperate and boreal sites were found to consume significantly greater amounts of CH_4 than tropical sites ($P < 0.010$). Natural land consumed more CH_4 than managed land ($P < 0.001$). The type of fertilizer used significantly affected CH_4 flux ($P < 0.021$); sites fertilized with NPK fertilizer mixtures were found consume more CH_4 than sites treated with urea, though neither was different from other N fertilizer forms. Long vs. short-term duration of fertilization had no effect ($P < 0.666$). Sites that were fertilized during the study consumed less CH_4 than those only fertilized before ($P > 0.001$).
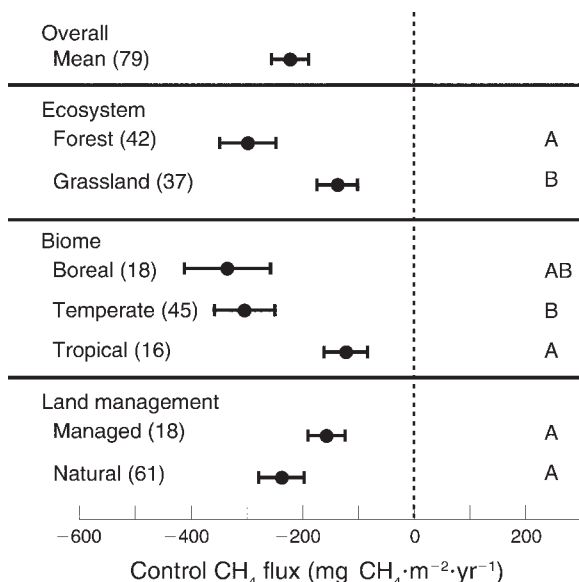
FIG. 7. Categorical variable effects on control CH_4 flux in control plots. Values expressed are means bounded by the standard error of the mean, with sample size indicated in parentheses. Different letters indicate statistically significant differences between means (Tukey's hsd, $P < 0.05$). The dotted line is zero on the x-axis.

DISCUSSION

Our analysis supports the hypothesis that N addition decreases CH_4 uptake in non-wetland ecosystems (Liu and Greaver 2009). However, this trend is not consistent across studies, indicating that the interaction between N inputs and soil CH_4 flux is more complicated than this larger trend. Across sites, many factors appear to affect CH_4 flux and its response to N addition, including: ecosystem, biome, temperature, precipitation, latitude, amount and type of N added, duration of N addition, and previous land use.

Overall, longer duration of fertilization decreased soil CH_4 uptake with N addition relative to the control during the study period. A similar trend was seen in that those sites classified as managed showed a stronger response to N added than natural sites. Natural and/or short-term fertilized soils were less likely to have a positive T value than those that had been managed or fertilized before. In particular, the amount of N added to plots that were previously N amended or managed showed a significant correlation with the study index. This strongly suggests that the historical N status of soil is the most important predictor of how the soil will respond to future inputs of nitrogen. In addition, the "grassland" classification in the meta-data set included cropped systems where the natural state of the land was grassland or where it was not reported. Therefore the greater T value in grasslands vs. forest could also be a result of the management of the land, not an actual difference between the ecosystems.

The microbial oxidation of CH_4 necessitates available oxygen, N and CH_4 in any environment. The nutrient limitation hypothesis (Tilman 1985) predicts that whichever of these is in shortest supply within a given environment is the "limiting" compound. When this limiting element or compound is added, it would logically increase the amount of CH_4 oxidation that can be performed, until saturation. If the ratio of existing available inorganic N to available CH_4 to which methanotrophs have access determines the rate of CH_4 oxidation of the system, then when this ratio is low any addition of N may stimulate increased CH_4 oxidation. Recent studies by Bodelier et al. (2000) have shown in rice systems severely limited by inorganic N that there is very little, if any, activity of methanotrophs and that increasing N addition in these soils allows for the oxidation of more CH_4. One proposed explanation for this N limitation is that when abundant ammonia is not available, methanotrophs must perform N fixation (Bodelier and Laanbroek 2004), which is energetically costly.
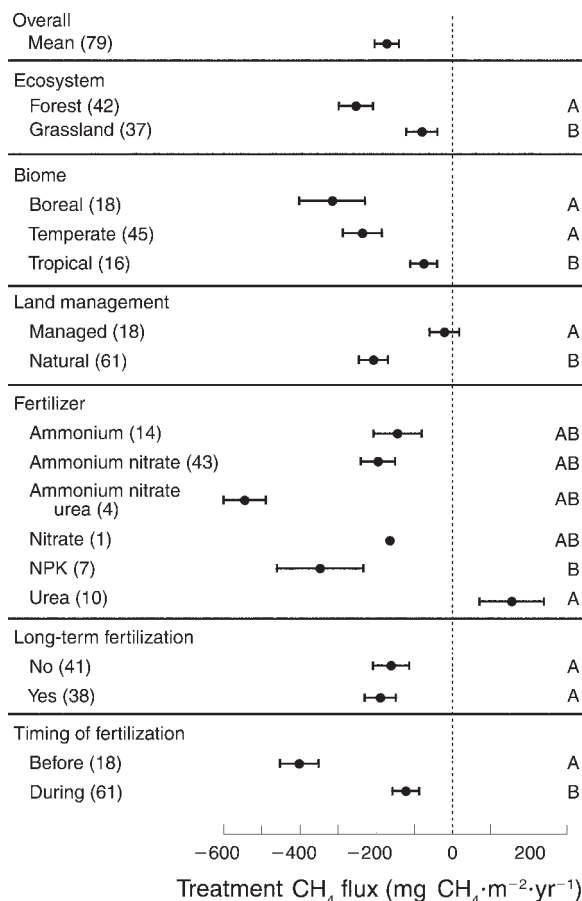

FIG. 8. Categorical variable effects on control CH_4 flux in treated plots. Values expressed are means bounded by the standard error of the mean, with sample size indicated in parentheses. Different letters indicate statistically significant differences between means (Tukey's hsd, $P < 0.05$). The dotted line is zero on the x-axis.

If the N status of managed lands, such as those in studies included in this meta-analysis were chronically increased, and if this N were not entirely leached out of the system, this would increase the existing ratio of available N to available atmospheric CH$_4$ in the soil. This could lead to a strong decrease in CH$_4$ uptake with N over-saturation due to previously mentioned enzymatic switching to ammonium oxidation. This hypothesis is supported by the fact that N-amended natural sites consumed more CH$_4$ than N-amended managed sites. It has been shown that the actual duration of agricultural use has a large impact on existing N status (Burchard 1998). Thus, this meta-analysis result further strengthens the assertion that in relatively N limited systems, here represented by those with shorter term fertilization soils, N addition may have a stimulatory effect on CH$_4$ uptake, rather than a deleterious one (Bodelier and Laanbroek 2004).

Nitrogen saturation in this system would correspond to the switch from stimulation of CH$_4$ consumption, when N is added to soil containing lower amounts of N, to inhibition with higher amounts. In this analysis, when all other variables were accounted for, that switch occurred at around 100 kg N·ha^{-1}·yr^{-1}. The average available wet N deposition on the Earth's terrestrial surface is greater than 64 Tg N/yr (Galloway et al. 2004). This translates to more than 4.6 kg N·ha^{-1}·yr^{-1}, and currently rising, when averaged over the total Earth land surface of about 1.4×10^{10} ha (Potter et al. 1996). However, deposition is not uniform, and in many places, in Europe in particular, it approaches the experimentally significant rate of 100 kg N·ha^{-1}·yr^{-1}. In areas of lower N deposition, the addition of fertilizer easily raises the total added available N above this amount. Therefore, small changes in rates of deposition or fertilization can lead to significant changes in the methane consumption potential of soil and can even reverse the direction of flux.

A few of the papers described in this meta-analysis tested the effects of multiple levels of N on CH$_4$ flux. Their results support the conclusion that the ratio of available N to CH$_4$ in the soil may be the main determinant of CH$_4$ flux. Kruse and Iversen (1995) saw an increase in CH$_4$ uptake with the addition of 56 kg N·ha^{-1}·yr^{-1} in a heath site but a decrease in CH$_4$ uptake with 112 kg N·ha^{-1}·yr^{-1}. Ambus and Robertson (2006) used two levels of N on coniferous and deciduous forest soils, 10 and 30 kg N·ha^{-1}·yr^{-1}, and saw a trend of increasing CH$_4$ uptake with greater N addition in the first year. After continued N addition, the trend reversed (Ambus and Robertson 2006), with soil inorganic N inversely proportional with the CH$_4$ uptake of the soil, possibly due to accumulation from the previous year. Ding et al. (2004) reported on an agricultural site that had been fertilized at three different levels (9, 19, and 23 kg N·ha^{-1}·yr^{-1}) and reported a direct correlation after five years between fertilizer application and increased NH$_4$ concentration in the surface soil, and an inverse relationship between these and CH$_4$ uptake.

Another possibility is that the effects of N limitation on methanotrophy may be indirect, acting though the plants that provide carbon compounds to the soil via the root-priming effect. Consider that the methanotrophs present and active in non-wetland systems are performing methanotrophy at atmospheric CH$_4$ levels (Kolb 2009). No organism has ever been cultured that subsists on CH$_4$ at low concentrations without another source of energy, such as acetate in *Methylocella* sp. (Conrad 2009). Therefore the methanotrophs responsible are likely facultative in their consumption of atmospheric CH$_4$, and are more likely to replicate when the N limitation on non-methanotrophic growth is alleviated. This replication would lead to a greater number of cells present in the soil capable of CH$_4$ consumption at the same rate as previous to N addition, rather than causing individual cells to increase their methane consumption rate, as the above hypothesis posits. If these facultative methanotrophs are involved in the consumption of plant-derived carbon compounds via root exudation (Kuzyakov et al. 2000), and the plants in the system respond to the increase in available N by decreasing the carbon compounds they leach into the system, the decreased availability of plant-derived substrate could cause the larger group of methanotrophs now present in the system to perform methanotrophy at a higher rate.

Alternatively, when Chan and Parkin (2001) found that soil incubated at high [CH$_4$], CH$_4$ oxidation increased directly with inorganic N concentration, the authors attributed the pattern to different communities of methanotrophs in different sites. It is impossible to determine whether the major trends observed in this analysis are due to N limitation and saturation, as previously discussed, or to the cultivation of different microbial consortia in soils exposed to chronic N addition. As the soils explored by each of the studies included in this analysis were either managed or unmanaged previous to experimental manipulation (to limit variation external to the target effect), further study must be performed on this topic using paired sets of plots under similar climates with different management regimes if a conclusion is to be drawn on this topic.

The type of N fertilizer used affected the outcome of fertilization. Nitrate was found to yield the greatest decrease in CH$_4$ uptake (positive T), followed by urea. On average, ammonium by itself (i.e., no other N fertilizers added) had the unexpected effect of increasing the CH$_4$ uptake of the sites. This could be due to the fact that the ammonium ion was added as ammonium phosphate, ammonium sulfate, or some other compound, and in fact the non-nitrogen component was having the stimulatory effect on CH$_4$ flux. The amount of N being added may also affect this trend, as the average amount of ammonium added was less than half of the amount of urea added. However the amount of ammonium was comparable to the amount of nitrate added, so this cannot completely explain the discrepancies. The effects of N addition could be similar

regardless of N form used, due to the presence of microorganisms capable of rapid N transformation by variation in microbial consortia. The timing of fertilization may determine the form of N that methane-cycling microorganisms encounter in the soil more than the actual N species added. The fact that the addition of urea and ammonium nitrate were capable of increasing nitrate availability significantly in Delgado et al. (1996) underscores this point. Therefore, any conclusions of the effects of specific N species relative to others must be highly qualified, as the form of N that results may be quite different from that added.

All explanations offered in the preceding paragraphs rely on N treatment primarily affecting the methanotrophic community in the soil because that is where the most plausible mechanisms of action exist. It is unlikely that the decrease in soil CH_4 consumption with N addition is actually due to an increase in methanogenesis. Increasing available ammonium and nitrate, due to soil N additions, would decrease macromolecular (e.g., lignin) decomposition (Lucas and Casper 2008). This in turn would decrease microbial access to the smaller organic compounds that are precursors to anaerobic decomposition, including methanogeny. This would increase soil consumption with higher ammonium additions, which is not the case. There is some evidence that nitrate inhibits methanogenesis in soils (Balderston and Payne 1976). However, Neff et al. (1994) found that there was no relationship between the concentration of ammonium in the soil and CH_4 uptake, but that net nitrification (microbial oxidation of ammonia to nitrite) correlated with decreased CH_4 uptake, which is the opposite response.

Greater soil moisture, which can be correlated with precipitation, is generally regarded as the greatest predictor of CH_4 flux (Le Mer and Roger 2001). Precipitation was a predictor of CH_4 flux in treatment, but not control, plots. In seasonal tropical forest, a high precipitation ecosystem, precipitation exclusion has increased uptake of CH_4 (Davidson et al. 2008). The dampening of CH_4 consumption by addition of large amounts of N may have worked in tandem with the stimulation of CH_4 generation from greater precipitation inputs, which may explain the lack of precipitation trend in control plots and the presence of this trend in treatment plots.

This analysis suggests that CH_4 consumption is lower in higher temperatures (and in the tropical biome), which has obvious impacts for predicted effects of climate change, but that N addition has a stronger inhibitive effect in colder temperatures. Perhaps the lower level of atmospheric CH_4 consumption in higher temperatures (and in the temperate zone) means that any N inhibition is too subtle to be significant or that consumption is already below a threshold for minimum CH_4 consumption. Latitude is often used as a proxy for temperature, however they did not correlate in this study. Latitude may therefore have instead been a proxy for macrobiotic

diversity gradients (Fischer 1960). This would indicate that the lack of effect of latitude on any variables in this study means that these larger diversity gradients have no effect on microbial activity, though this is an area of study that deserves more direct attention.

CONCLUSIONS

While measurements of CH_4 uptake vary considerably within and across sites, our meta-analysis shows that several broad generalizations can be made. Nitrogen decreases the CH_4 sink capacity of non-wetland ecosystems in most cases. Lower levels of fertilization can stimulate CH_4 uptake in the soil, while higher fertilization rates incur a decrease in uptake. Soils that are pre-exposed to N fertilization are affected more by subsequent N fertilization events than pristine non-impacted soils. The historical N status of soils is therefore an important input parameter for models when the effect of N deposition or fertilization is predicted. The CH_4 sink capacity of colder soils, and that of systems which receive a larger amount of rainfall, are more likely to shrink in response to N addition. The incorporation of these findings into global climate models may yield a more accurate representation of the interactions between the N and carbon cycles, and better predictions of future climatic feedback trends.

LITERATURE CITED

Ambus, P., and G. P. Robertson. 2006. The effect of increased n deposition on nitrous oxide, methane and carbon dioxide fluxes from unmanaged forest and grassland communities in Michigan. Biogeochemistry 79:315–337.

Arnqvist, G., and D. Wooster. 1995. Meta-analysis: synthesizing research findings in ecology and evolution. Trends in Ecology and Evolution 10:236–240.

Balderston, W. L., and W. J. Payne. 1976. Inhibition of methanogenesis in salt-marsh sediments and whole-cell suspensions of methanogenic bacteria by nitrogen-oxides. Applied and Environmental Microbiology 32:264–269.

Bodelier, P. L. E., and H. J. Laanbroek. 2004. Nitrogen as a regulatory factor of methane oxidation in soils and sediments. FEMS Microbiology Ecology 47:265–277.

Bodelier, P. L. E., P. Roslev, T. Henckel, and P. Frenzel. 2000. Stimulation by ammonium-based fertilizers of methane oxidation in soil around rice roots. Nature 403:421–424.

Bousquet, P., et al. 2006. Contribution of anthropogenic and natural sources to atmospheric methane variability. Nature 443:439–443.

Bradford, M. A., P. Ineson, P. A. Wookey, and H. M. Lappin-Scott. 2001. The effects of acid nitrogen and acid sulphur deposition on CH4 oxidation in a forest soil: a laboratory study. Soil Biology and Biochemistry 33:1695–1702.

Burchard, I. 1998. Anthropogenic impact on the climate since man began to hunt. Palaeogeography Palaeoclimatology Palaeoecology 139:1–14.

Chan, A. S. K., and T. B. Parkin. 2001. Methane oxidation and production activity in soils from natural and agricultural ecosystems. Journal of Environmental Quality 30:1896–1903.

Christensen, T. R., A. Michelsen, and S. Jonasson. 1999. Exchange of CH4 and N2O in a subarctic heath soil: effects of inorganic N and P and amino acid addition. Soil Biology and Biochemistry 31:637–641.

Conrad, R. 2009. The global methane cycle: recent advances in understanding the microbial processes involved. Environmental Microbiology Reports 1:285–292.

Davidson, E. A., D. C. Nepstad, F. Y. Ishida, and P. M. Brando. 2008. Effects of an experimental drought and recovery on soil emissions of carbon dioxide, methane, nitrous oxide, and nitric oxide in a moist tropical forest. Global Change Biology 14:2582–2590.

Delgado, J. A., A. R. Mosier, R. H. Follett, R. F. Follett, D. G. Westfall, L. K. Klemedtsson, and J. Vermeulen. 1996. Effects of N management on N$_2$O and CH$_4$ fluxes and N-15 recovery in an irrigated mountain meadow. Nutrient Cycling in Agroecosystems 46:127–134.

Ding, W. X., Z. C. Cai, and H. Tsuruta. 2004. Cultivation, nitrogen fertilization, and set-aside effects on methane uptake in a drained marsh soil in Northeast China. Global Change Biology 10:1801–1809.

Dumont, M. G., and J. C. Murrell. 2005. Stable isotope probing: linking microbial identity to function. Nature Reviews Microbiology 3:499–504.

Dunfield, P., and R. Knowles. 1995. Kinetics of inhibition of methane oxidation by nitrate, nitrite and ammonium in a humisol. Applied and Environmental Microbiology 61:3129–3135.

Dutaur, L., and L. V. Verchot. 2007. A global inventory of the soil CH$_4$ sink. Global Biogeochemical Cycles 21:GB4013.

Fischer, A. G. 1960. Latitudinal variations in organic diversity. Evolution 14:64–81.

Galloway, J. N., et al. 2004. Nitrogen cycles: past, present, and future. Biogeochemistry 70:153–226.

Gulledge, J., A. P. Doyle, and J. P. Schimel. 1997. Different NH$_4^+$-inhibition patterns of soil CH$_4$ consumption: a result of distinct CH$_4$-oxidizer populations across sites? Soil Biology and Biochemistry 29:13–21.

Hanson, R. S., and T. E. Hanson. 1996. Methanotrophic bacteria. Microbiological Reviews 60:439–471.

Holmes, A. J., P. Roslev, I. R. McDonald, N. Iversen, K. Henriksen, and J. C. Murrell. 1999. Characterization of methanotrophic bacterial populations in soils showing atmospheric methane uptake. Applied and Environmental Microbiology 65:3312–3318.

Hutchinson, G. L., and A. R. Mosier. 1981. Improved soil cover method for field measurement of nitrous-oxide fluxes. Soil Science Society of America Journal 45:311–316.

Kolb, S. 2009. The quest for atmospheric methane oxidizers in forest soils. Environmental Microbiology Reports 1:336–346.

Kroeze, C., and S. P. Seitzinger. 1998. Nitrogen inputs to rivers, estuaries and continental shelves and related nitrous oxide emissions in 1990 and 2050: a global model. Nutrient Cycling in Agroecosystems 52:195–212.

Kruse, C. W., and N. Iversen. 1995. Effect of plant succession, plowing, and fertilization on the microbiological oxidation of atmospheric methane in a heathland soil. FEMS Microbiology Ecology 18:121–128.

Kuzyakov, Y., J. K. Friedel, and K. Stahr. 2000. Review of mechanisms and quantification of priming effects. Soil Biology and Biochemistry 32:1485–1498.

Lelieveld, J., P. J. Crutzen, and F. J. Dentener. 1998. Changing concentration, lifetime and climate forcing of atmospheric methane. Tellus Series B-Chemical and Physical Meteorology 50:128–150.

Le Mer, J., and P. Roger. 2001. Production, oxidation, emission and consumption of methane by soils: a review. European Journal of Soil Biology 37:25–50.

Liu, L. L., and T. L. Greaver. 2009. A review of nitrogen enrichment effects on three biogenic GHGs: the CO$_2$ sink may be largely offset by stimulated N$_2$O and CH$_4$ emission. Ecology Letters 12:1103–1117.

Lucas, R. W., and B. B. Casper. 2008. Ectomycorrhizal community and extracellular enzyme activity following simulated atmospheric N deposition. Soil Biology and Biochemistry 40:1662–1669.

Neff, J. C., W. D. Bowman, E. A. Holland, M. C. Fisk, and S. K. Schmidt. 1994. Fluxes of nitrous-oxide and methane from nitrogen-amended soils in a Colorado alpine ecosystem. Biogeochemistry 27:23–33.

Ojima, D. S., D. W. Valentine, A. R. Mosier, W. J. Parton, and D. S. Schimel. 1993. Effect of land-use change on methane oxidation in temperate forest and grassland soils. Chemosphere 26:675–685.

Potter, C. S., E. A. Davidson, and L. V. Verchot. 1996. Estimation of global biogeochemical controls and seasonality in soil methane consumption. Chemosphere 32:2219–2246.

Rigby, M., et al. 2008. Renewed growth of atmospheric methane. Geophysical Research Letters 35:L22805.

Rustad, L. E., J. L. Campbell, G. M. Marion, R. J. Norby, M. J. Mitchell, A. E. Hartley, J. H. C. Cornelissen, and J. Gurevitch. 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. Oecologia 126:543–562.

Schaefer, H., and M. J. Whiticar. 2008. Potential glacial-interglacial changes in stable carbon isotope ratios of CH$_4$ sources and sink fractionation. Global Biogeochemical Cycles 22:GB1001.

Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, and H. L. Miller, editors. 2007. Climate change 2007: the physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.

Tilman, D. 1985. The resource-ratio hypothesis of plant succession. American Naturalist 125:827–852.

Willison, T. W., K. W. T. Goulding, and D. S. Powlson. 1995. Effect of land-use change and methane mixing ration on methane uptake from United Kingdom soil. Global Change Biology 1:209–212.

Zhuang, Q., J. M. Melillo, D. W. Kicklighter, R. G. Prinn, A. D. McGuire, P. A. Steudler, B. S. Felzer, and S. Hu. 2004. Methane fluxes between terrestrial ecosystems and the atmosphere at northern high latitudes during the past century: a retrospective analysis with a process-based biogeochemistry model. Global Biogeochemical Cycles 18:GB3010.

APPENDIX A

A list of papers from which data were extracted for this metadata analysis (*Ecological Archives* E091-228-A1).

APPENDIX B

Effects of continuous factors on CH$_4$ flux in control and nitrogen-amended plots (*Ecological Archives* E091-228-A2).