Methane flux response to nitrogen amendment in an upland pine forest soil and riparian zone

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[1] Methane (CH$_4$) is an important anthropogenic greenhouse gas, up to 15% of which is consumed by terrestrial soils. In this field study of the CH$_4$ cycle of a pine forest, 18 plots were established at each of two sites, located 40 m apart. The upper site was well-drained and the lower site was poorly drained, but they shared similar overstory tree composition. Nitrogen was added as NH$_4$NO$_3$ incrementally across the 2009 growing season in a high (67 kg NH$_4$NO$_3$ ha$^{-1}$ yr$^{-1}$) and a low (5 kg NH$_4$NO$_3$ ha$^{-1}$ yr$^{-1}$) concentration. The sites were monitored for soil and air temperature, soil moisture, precipitation, air pressure, and NH$_4$ and NO$_3$ concentrations throughout the growing season. Across all treatments for the duration of the field season, average CH$_4$ flux showed consumption of $-0.84$ kg CH$_4$ ha$^{-1}$ yr$^{-1}$, but CH$_4$ flux differed between the upper and lower sites. Across all treatments, upper site CH$_4$ flux averaged $-5.38$ kg CH$_4$ ha$^{-1}$ yr$^{-1}$, while lower site flux averaged $3.72$ kg CH$_4$ ha$^{-1}$ yr$^{-1}$, with greater variance than was observed at the upper site. High N treatments caused greater CH$_4$ release than the control in the lower, but not the upper, site. The main correlated variable with CH$_4$ flux was soil moisture; however, it accounted for <14% of the variation. Statistics were run several different ways, resulting in multiple environmental factors contributing up to 84% of the variation in CH$_4$ flux. Long-term drainage differences between the sites likely drove the differences in CH$_4$ flux.


1. Introduction

[2] Methane (CH$_4$) is considered the third most potent greenhouse gas in terms of atmospheric heat-holding capacity [Intergovernmental Panel on Climate Change (IPCC), 2007]. Over the past 150 years, atmospheric CH$_4$ has increased monotonically due to anthropogenic inputs to the atmosphere. The increase in atmospheric CH$_4$ is believed to have been steady for much of that time period, but it became erratic in the 1990s and did not increase overall from around 1999 until 2008, when it began increasing again [Rigby et al., 2008]. The causes of these dramatic shifts are still hotly debated [Aydin et al., 2011; Kai et al., 2011].

[3] Drivers of variation in atmospheric CH$_4$ concentrations, in particular the controls on interannual changes in CH$_4$ flux into and out of soils, are only recently coming into focus. Field research in CH$_4$ flux has given detailed but somewhat conflicting views on the driving factors [McLain et al., 2002; Phillips et al., 2001]. The study of the microbial mechanisms involved is needed to address this uncertainty [Aronson and Helliker, 2010]. As soil microorganisms are the most important biological sources and sinks for CH$_4$, the study of highly complex in situ CH$_4$ flux responses to environmental stimuli can greatly improve our understanding of past, current and future fluctuations in atmospheric CH$_4$.

[4] Soil exchange of CH$_4$ with the atmosphere is regulated by two main groups of microorganisms, methanogens and methanotrophs. The different environmental requirements of these two groups, particularly oxygen level, water content, nutrient availability, and temperature, determine the CH$_4$ flux of a given ecosystem [Silver et al., 1999]. Methanogenic archaea, active in anaerobic conditions, produce CH$_4$ as a byproduct of metabolism and are the main biological source of CH$_4$ in natural systems, landfills and agriculture [Bartlett and Harriss, 1993]. Methanotrophic bacteria are active in aerobic conditions and derive energy and carbon from the oxidation of CH$_4$ [Hanson and Hanson, 1996]. The balance between production and consumption by these groups determines the direction and magnitude of flux of CH$_4$ across the soil surface. Variation in temperature, precipitation, nitrogen status and other environmental parameters controls the balance of activity between methanogens and methanotrophs—and hence net CH$_4$ flux—in any soil [Conrad, 2007]. In particular, forest soil...
pH, forest type and temperature have been found to shape methanotrophic communities, while specific effects on individual microbial species are largely unknown [Kolb, 2009].

[5] Patterns of temperature and rainfall are shifting [IPCC, 2007], and changes in both can have dramatic impacts on CH4 uptake by soils [Le Mer and Roger, 2001]. Soil moisture, which is correlated both with precipitation and water table height, is generally regarded as the greatest predictor of CH4 flux. Precipitation exclusion has been shown to result in increased uptake of CH4 in seasonal tropical forest [Davidson et al., 2008], and heavy precipitation leads to decreased uptake or even release from terrestrial ecosystems [Singh et al., 1997]. In general, high and low temperatures inhibit CH4 consumption, while CH4 production correlates positively with increasing temperatures [Conrad, 2007; Castro et al., 1995]. Increased CO2 concentrations at forest Free Air CO2 Experiment (FACE) sites have been shown to decrease net methane consumption [Phillips et al., 2001], possibly due to increased soil moisture effects on methanogenesis in the lower soil layers [McLain et al., 2002; McLain and Ahmann, 2008; Dubbs and Whalen, 2010].

[6] Overall, nitrogen (N) input from atmospheric deposition and fertilizer use is projected to double from 1990 levels by the year 2050 [Kroeze and Seitzinger, 1998]. It has been established that cropland and pasture where N fertilizer is routinely applied tend to consume less CH4 than natural forest and grassland [Ojima et al., 1993; Willison et al., 1995]. In general this is thought to occur because added ammonium to the soil can lead to increased concentrations of ammonia, which many methanotrophs can oxidize in place of CH4 [Hanson and Hanson, 1996]. The genetics and enzyme kinetics behind CH4 oxidation show tight evolutionary and functional linkages between the enzymes that enable CH4 and ammonia oxidation [Dunfield and Knowles, 1995]. However, wetland research has shown that small amounts of N addition to nutrient-poor wetlands can result in increased CH4 oxidation, which may lower the net release of CH4 from the soil [Bodelier et al., 2000]. A recent meta-analysis on the impact of N addition on methane consumption in non-wetland ecosystems found that larger N additions led to decreased CH4 consumption from the atmosphere, and that previous fertilizer application to soil led to a greater decrease in CH4 consumption with new fertilization [Aronson and Helliker, 2010].

[7] In recent years there has been increasing interest in the study of the effects of environmental and climatic variables on CH4 consumption in forested ecosystems, which is one of the greatest but most variable biological sinks for CH4 [DuRant and Vrechot, 2007]. In particular, the Pinelands in New Jersey constitute the largest contiguous region of pine forest, a common vegetation type in the northeast USA, in the country [McCormick and Forman, 1979]. In the present study we characterize the effects of N addition, and the interactive effects of N addition with season, drainage, temperature and moisture, on the CH4 uptake and release of a sandy pine forest soil.

2. Materials and Methods

2.1. Site Description

[8] Measurements were made in a sandy pine forest soil in New Jersey, USA (39°55’N, 74°35’W) during the summer and early fall of 2009. The average annual temperature is 12.3°C and precipitation is 1143 mm. The sites were located roughly 1 mi from the Rutgers Pinelands Field Station, part of the greater New Jersey Pinelands Preserve, which includes approximately 304,000 ha of land with heavily restricted development in south-central New Jersey [Krumins et al., 2009]. The study location consisted of two sites separated by 30–40 m: one well-drained at an elevation of 30 m asl and 7 m above the water table. The other, poorly drained at an elevation of 23 m asl and located at the water table ±5 cm. The well-drained site was considered an “excessively drained” sand of the Evesboro series, which consists of Mesic, coated Lamellic Quartzipsamments covered by an organic (O) horizon [Krumins et al., 2009]. The poorly drained site was considered a Fluvuvaqent or “frequently flooded sediment” by the Web Soil Survey available from the U.S. Department of Agriculture (http://websoilsurvey.nrcs.usda.gov/app/), which in this case has a large O horizon of variable depth, sometimes topped with peat.

[9] The sites, though very different in soil and drainage characteristics, shared overstory species of trees. These included bear oak (Quercus ilicifolia), chestnut oak (Q. prinus), eastern black oak (Q. velutina), shortleaf pine (Pinus echinata) and pitch pine (P. rigida). The understory was more variable, and included lowbush blueberries (Vaccinium angustifolium), wintergreen (Gaultheria procumbens) and peat moss (Sphagnum spp.) among sparse grasses. Further information on vegetation in the New Jersey Pinelands has been published by Krumins et al. [2009].

[10] The New Jersey coastal forest is in the center of recent and predicted urban development, and is therefore in a zone of increasing nitrogen deposition [Holland et al., 2005]. The New Jersey Pinelands are broadly representative of coastal pine systems throughout the Southeastern USA. Thus, local results can be extrapolated to a large area of the United States, with analogues across the Earth. In this system, small-scale variations in water table and organic matter content lead to large variations in soil characteristics such as water holding capacity and oxygen content, which may alter the microbial composition, abundance and function. This system contains both wetland and upland forests while maintaining a mostly homogeneous overstory vegetation distribution, hence minimizing plant effects on microbial abundance and function. In particular, the Pinelands’ current rate of atmospheric nitrogen deposition [Dighton et al., 2004]. The high nitrogen level was intended to mimic the average level of fertilization in a cranberry bog.
Table 1. Incremental Amounts of N and H\textsubscript{2}O Added to Field Plots\textsuperscript{a}

<table>
<thead>
<tr>
<th>Nitrogen Level</th>
<th>Nitrogen Simulation</th>
<th>NH\textsubscript{4}NO\textsubscript{3} Level Annually (kg N ha\textsuperscript{-1} yr\textsuperscript{-1})</th>
<th>NH\textsubscript{4}NO\textsubscript{3} Added Every 3 Weeks (mg m\textsuperscript{-2})</th>
<th>H\textsubscript{2}O Added Every 3 Weeks per Plot (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Natural deposition</td>
<td>0</td>
<td>0 (half of plots) or 0 (half of plots)</td>
<td>1</td>
</tr>
<tr>
<td>Low N</td>
<td>Double deposition</td>
<td>5</td>
<td>164.8</td>
<td>1</td>
</tr>
<tr>
<td>High N</td>
<td>Fertilization</td>
<td>67</td>
<td>2208.8</td>
<td>1</td>
</tr>
</tbody>
</table>

\textsuperscript{a}The annual N quantities presented extrapolated to an annual rate, while N was added for a total of 144 days.

The most common form of agriculture in the New Jersey Pinelands [Davenport and Schiffauer, 2007].

Half of the plots had been established for a trial run of the design in May 2008, including nine upland and nine lowland plots, of which each of the three treatments had been replicated three times. In 2008, two fertilizer applications of 736.25 mg m\textsuperscript{-2} for high N and 1 mg m\textsuperscript{-2} for low N were applied within a two-month period from mid-June through mid-August. In 2008, CH\textsubscript{4} flux measurements were made and some environmental variables were monitored. These data were used to assess the power of the research plan to establish the larger investigation in 2009, and these data are not reported. The nine newer plots in both the upland and lowland sites were established in late April 2009, and fertilizer applications began on 8 June 2009, with each of the plots established in 2008 receiving the same N treatment (high, low or no N) as in the previous year. The variable “condition” was used in subsequent statistical analysis to connote whether the plot was established in 2008 (“old”) or in 2009 (“new”).

Soil sampling in a given week was performed on a block of six upper and six lower randomly selected plots, on the day before, the day of, and two days after fertilization, called “pre-fertilization,” “fertilization day,” and “post-fertilization,” respectively. The plots were then left fallow for the next two weeks, while the other two blocks of 12 plots were analyzed. Time domain reflectometry (TDR) sensors (CS616, Campbell Scientific) and thermocouples were implanted randomly across the upper and lower sites and connected to a CR-1000 data logger (Campbell Scientific) for constant measurement of environmental variables between plots in the two sites. In July 2009, three soil oxygen probes (SO-110, Apogee Instruments) were implanted randomly in each of the upper and lower sites.

2.3. Gas Flux Measurements

Gas was collected for CH\textsubscript{4} flux analysis in situ using opaque, nonreflective PVC chamber bases, with an internal diameter of 30 cm and a height of 20 cm. These were implanted randomly in the central 1 \times 1 m of each plot, approximately 5 cm into the soil, resulting in an internal volume above the soil surface of approximately 10.5 L, as described by Neff et al. [1994]. The chamber bases were in place at least two weeks before measurements started and remained in place throughout the summer and fall. During gas sampling, the chamber bases were fitted with a removable, opaque, nonreflective, flat PVC cover with a rubber septum port for sampling as well as a port fitted with 4 mm diameter tubing for pressure compensation, as described by Livingston and Hutchinson [1995]. The chambers were sealed with nonreactive vacuum grease (Dow Corning, Inc.), as described by Nkongolo et al. [2010]. This vacuum grease sealing method was lab tested, and was found to create an effective seal while having no effect on CH\textsubscript{4} flux rates, comparable with using water as a sealant (unpublished data). Water was determined unsuitable as a sealant in the field due to the risk of water spilling into the plot treatment area, thereby changing the soil moisture content during gas flux analysis, possibly impacting the CH\textsubscript{4} flux result.

Gas was collected for analysis at three time points, in Tedlar bags filled using a vac-u-tube (SKC Inc.) fitted with a needle, every 15 min for a total of 30 min. Tests showed that the concentration of CH\textsubscript{4} inside the Tedlar bags remained unchanged for at least three weeks (data not shown). These bags were usually analyzed within one week of sampling, and the longest they were stored before analysis was two weeks. Tedlar bags were chosen for sampling rather than the more commonly used syringe to vial collection method, because both evacuated and noble gas-filled exetainers fitted with rubber septa were found to contribute CH\textsubscript{4} to the samples.

Methane concentration was analyzed by gas chromatograph with attached flame ionization detector (GC-FID) (Trace GC, Thermo Instruments Inc.). The FID temperature was 190°C, and a Porapak Q column (Varian Inc.) of 25 m and 0.32 mm ID was used at 45°C. The carrier gas was He at a maximum of 10 ml min\textsuperscript{-1}. Since the changes in CH\textsubscript{4} concentrations were generally linear with time, the fluxes were calculated using linear regression and the ideal gas law [Livingston and Hutchinson, 1995]. Net CH\textsubscript{4} consumption by the soil was expressed using negative numbers, while positive numbers were used to express net release by the soil into the atmosphere.

2.4. Soil Analyses

During gas collection, instantaneous moisture and temperature measurements were taken of the top 5 cm of the soil in each plot, just outside the chamber base, by a 5TE probe (Decagon Devices). Relative humidity, pressure and precipitation were measured hourly at a nearby Forest Service eddy covariance tower; more information is provided by Clark et al. [2010]. Soil water potentials were computed from pedo-transfer functions using observed soil moisture measurements as described by Vereecken et al. [1989]. This equation, modified from its original version from van Genuchten [1980] was evaluated by Cornelis et al. [2001] to be the best fit pedo-transfer function in the literature based on a large variety of soils. As the Pinelands soils are composed of sand and organic matter (see site description), the percentage of sand for the pedo-transfer function was assumed to be the remainder when the % C and N were subtracted.

Soil cores (5 cm ID and 30.5 cm long) and adjacent soil from the top 5 cm were collected on gas-sampling dates.
Topsoil was extracted within a few hours with KCl for NH$_4^+$ and NO$_3^-$+NO$_2^-$. Adjacent soil was weighed before and after oven drying to produce gravimetric water content for the analysis of NH$_4^+$ and NO$_3^-$/NO$_2^-$ data. Extracts were analyzed colorimetrically with an Astoria-Pacific AutoAnalyzer 3 (Clackamus, Oreg.). The indophenol method was used to analyze NH$_4^+$ [Standard Methods Committee, 1976] and NO$_3^-$/NO$_2^-$ was analyzed according to the N-1-naphthylenediamine dihydrochloride (NED) method following hydrazine reduction [Environmental Monitoring and Support Laboratory, 1983].

[19] The soil % content of N and C were analyzed at the start and the end of the growing season by elemental analyzer (Carlo Erba Instrumentazione, SA, Milan, Italy, model NA1500). Soil N and C analysis was performed on dried soils, used for gravimetric water content measurement, from the first date of collection (“pre-fertilization”) and the last “fertilization day” of the field season in 2009.

[20] In July 2010, additional soil was collected from each plot for bulk density measurements. Bulk density was determined by removing and combining 3 identical cores with a corer of internal diameter 2.54 cm and length 15.24 cm. These were then dried in a drying oven at 110°C for 48 h and the dry weight determined. The bulk density was determined by dividing the dry weight by the volume of soil collected, which was 308.73 cc for each plot.

[21] Also in July 2010, a supplemental investigation was conducted of the treatment impacts on soil O$_2$ in the field site and in a growth chamber with soil removed from the field site. In the field, one O$_2$ sensor each was implanted at 5 cm depth into two lower site, high N plots. One plot was treated with high N while the other was watered. This effect was also measured in a climate-controlled growth chamber (Conviron, Inc.) simulating summer field conditions with soil collected from a water-saturated location 5 m from the lower site. Six pots were created out of opaque PVC pipe 25 cm ID and 60 cm long, with flat pieces of PVC cemented on one end. Pots were filled in the field with intact pillars of soil 30 cm deep, and transported back to the growth chamber without disturbance. Two pots were not amended, another two were watered to saturation, while a final two were watered to saturation with high N amendment. Each pot had an O$_2$ sensor implanted 5 cm into the soil continuously collecting O$_2$ data for 7 days, starting 3 h prior to amendment. A 5TE sensor was used to collect periodic temperature and moisture measurements throughout.

### Table 2. Soil Properties by Site Across All Treatments^a

<table>
<thead>
<tr>
<th>Environmental Variable</th>
<th>Sample Size (U, L)</th>
<th>Upper Site</th>
<th>Lower Site</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk Density (g cc$^{-1}$)</td>
<td>18, 18</td>
<td>1.37 ± 0.13</td>
<td>0.22 ± 0.24</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>% C content June</td>
<td>18, 18</td>
<td>23.77 ± 11.69</td>
<td>45.91 ± 1.94</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>% C content October</td>
<td>18, 18</td>
<td>9.36 ± 8.99</td>
<td>38.84 ± 9.2</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>% N content June</td>
<td>18, 18</td>
<td>0.86 ± 0.42</td>
<td>1.36 ± 0.2</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>% N content October</td>
<td>18, 18</td>
<td>0.29 ± 0.31</td>
<td>1.12 ± 0.41</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Soil Moisture (ml H$_2$O ml$^{-1}$ soil)</td>
<td>240, 240</td>
<td>0.13 ± 0.04</td>
<td>0.40 ± 0.15</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Soil water potential (MPa)</td>
<td>240, 240</td>
<td>−0.022 ± 0.017</td>
<td>−0.005 ± 0.0034</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Soil temperature (°C)</td>
<td>240, 240</td>
<td>25.61 ± 7.44</td>
<td>20.92 ± 4.57</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Soil NH$_4$ content (mg N g$^{-1}$ dry soil)</td>
<td>214, 216</td>
<td>21.21 ± 26</td>
<td>29.95 ± 41.38</td>
<td>&lt;0.0002*</td>
</tr>
<tr>
<td>Soil NO$_3$-N content (mg N g$^{-1}$ dry soil)</td>
<td>214, 216</td>
<td>4.67 ± 12.5</td>
<td>12.79 ± 30.02</td>
<td>&lt;0.0003*</td>
</tr>
</tbody>
</table>

^aMeans ± standard deviation are given along with significance levels of t-test comparisons. Soil temperature, moisture and NH$_4^+$ and NO$_3^-$/NO$_2^-$ content averages are from all dates/times of CH$_4$ flux measurements. Bulk density is given for the top 15 cm of soil, while all other measurements are for the top 10 cm of soil. The % N and C content are given for the very first pre-fertilization date of all plots (June) and for the last fertilization date of all plots (October). Asterisks next to p-values indicate significance at p < 0.05, and p-values given are for one-way t-test differences between the two sites. U, L = Upper Site, Lower Site.
In order to establish the rate of flux across time, all CH$_4$ flux measurements were evaluated by linear regression. In order to weight the measurements of CH$_4$ flux by the accuracy of the multiple measurements for each time point, a weight statistic $W$ was used in a subset of the statistical analyses. $W$ was calculated as the inverse of the standard error of the slope of the line of best fit of the linear regression for CH$_4$ flux for each ring.

3. Results

3.1. Environment and Soil Properties

[24] The average ± standard deviation of daily air temperature was 22.7 ± 5.5°C and total rainfall was 533 mm during the 144 day study period (see Figure 1 for variation in these parameters across time). Soil properties found to be significantly different between sites are described in Table 2 for all treatments. Significantly different soil properties include bulk density, soil moisture, water potential, temperature, NH$_4$ and NO$_2$+NO$_3$. The mean ± standard error of the soil moisture across the growing season of the upper site was 0.13 ± 0.04 ml H$_2$O ml$^{-1}$ soil, while the lower site had a moisture of 0.40 ± 0.15 ml H$_2$O ml$^{-1}$ soil.

[25] Overall, there was a decrease in both N and C between June and October (i.e., across time, $p < 0.0001$ in both cases), but this difference was more pronounced in the upper site, and the site term was also significant ($p < 0.0001$) for both % N and % C (see Table S1 in the auxiliary materials). Across the field season, N decreased 66.27% in the upper site and 17.65% in the lower site, and C decreased 60.62% in the upper site and 15.40% for the lower site.

3.2. CH$_4$ Flux Effects

[26] Across all plots for the duration of the field season, the average CH$_4$ flux was an unweighted $-9.58$ µg CH$_4$ m$^{-2}$ h$^{-1}$, weighted $-10.66$ µg CH$_4$ m$^{-2}$ h$^{-1}$. The average for all control plots across sites was $-18.21$ µg CH$_4$ m$^{-2}$

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1Auxiliary materials are available in the HTML. doi:10.1029/2012JG001962.

Figure 2. Averages by site bounded by the standard error of the mean of CH$_4$ flux across all plots, including treatments and controls, for the duration of the growing season. The values were significantly different at $p < 0.0001$. Sample size (n) for each site is 240 measurements.

Figure 3. Averages bounded by the standard error of the mean of CH$_4$ flux across all plots for the duration of the growing season, by N treatment for (a) upper and (b) lower sites. Different letters indicate differences in average values of CH$_4$ flux between treatments within each site by Tukey’s HSD. Each sample size (n) is 80 measurements.
Due to lost degrees of freedom, F tests were not possible for
within plot interactions. Univariate unadjusted epsilon
probability values were used instead, and all tests were sig-
nificant, including all within interactions (p < 0.0001), time
(p < 0.0001), time by site (p < 0.0001), time by N treatment
(p < 0.0010) and time by site by N treatment (p < 0.0485).

3.3. Oxygen Analysis

[30] The addition of N correlated with lower soil O2 con-
centrations in the field plots and growth chamber pots
studied, as analyzed using matched pairs t-tests. In the field,
the O2 sensors at 5 cm depth in the unfertilized plot found an
average of 21.11% of the volume of air, while those in the
fertilized plot were lower, at 20.52% (p < 0.0001). In the
laboratory growth chamber, the average soil O2 concen-
tration of the unamended (control) pots in the growth chamber
was 21.76% of the volume of air, the average of the watered
pots was 21.80%, and the average for the N-fertilized and
watered pots was 21.71%. The fertilized pots had significantly
lower O2 concentrations than the unamended pots (p < 0.029).

Table 3. Single Variable Linear Regression Intercepts and Coefficients of Variation (of the Form y = mx + b) for the Impact of Environmental Variables on CH4 Flux (μg CH4 m⁻² h⁻¹) of All Observations

<table>
<thead>
<tr>
<th>Environmental Variable</th>
<th>Slope (m)</th>
<th>Intercept (b)</th>
<th>Sample Size (n)</th>
<th>R² Value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk Density (g cc⁻¹)</td>
<td>-94.2 ± 12.7</td>
<td>65.6 ± 12.8</td>
<td>36</td>
<td>0.103</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>% C Content June</td>
<td>3.2 ± 0.6</td>
<td>-118.6 ± 21.6</td>
<td>36</td>
<td>0.062</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>% C Content October</td>
<td>3.0 ± 0.5</td>
<td>-80.2 ± 13.6</td>
<td>36</td>
<td>0.084</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>% N Content June</td>
<td>75.3 ± 19.9</td>
<td>-91.2 ± 23.5</td>
<td>36</td>
<td>0.030</td>
<td>&lt;0.0002*</td>
</tr>
<tr>
<td>% N Content October</td>
<td>86.2 ± 14.3</td>
<td>-69.8 ± 26.0</td>
<td>36</td>
<td>0.072</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Soil Moisture (ml H2O ml⁻¹ soil)</td>
<td>366.4 ± 44.0</td>
<td>-107.0 ± 14.0</td>
<td>480</td>
<td>0.127</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Soil Water Potential (MPa)</td>
<td>14963.8 ± 3688.9</td>
<td>124.5 ± 22.4</td>
<td>480</td>
<td>0.073</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Soil Temperature (°C)</td>
<td>-4.5 ± 1.2</td>
<td>96.0 ± 29.6</td>
<td>480</td>
<td>0.028</td>
<td>&lt;0.0002*</td>
</tr>
<tr>
<td>Soil NH4 Content (mg N g⁻¹ dry soil)</td>
<td>0.6 ± 0.3</td>
<td>-25.5 ± 11.2</td>
<td>430</td>
<td>0.013</td>
<td>&lt;0.0185*</td>
</tr>
<tr>
<td>Soil NO3 Content (mg N g⁻¹ dry soil)</td>
<td>0.9 ± 0.4</td>
<td>-17.8 ± 9.6</td>
<td>430</td>
<td>0.013</td>
<td>&lt;0.0196*</td>
</tr>
</tbody>
</table>

*aThe estimates are bounded by standard errors. Asterisks next to p-values indicate significance at p < 0.05.

Figure 4. Linear regression of soil moisture by CH4 flux across all plots and measurement dates in both sites. Soil moisture is given as the volume of water out of the total intact soil volume.
Figure 5. Average methane fluxes across time, pooled by fertilization stage for (a) upper and (b) lower sites. Dates of pre-fertilization are shown as “Prefert,” fertilization dates are shown as “Fert,” and post-fertilization dates are shown as “Postfert.” Asterisks indicate dates where CH$_4$ fluxes were significantly different between treatments.
with no significant difference between the control and watered pots O2 concentrations (p < 0.732) nor between the fertilized and watered pots (p < 0.141). There was no significant impact of treatment on CH4 flux in the growth chamber (p < 0.818). The average CH4 flux in growth chamber pots was −13.57 μg CH4 m−2 h−1 for control, −6.26 μg CH4 m−2 h−1 for watered pots, and −12.33 μg CH4 m−2 h−1 for N-fertilized and watered pots.

4. Discussion

[31] Methane flux across the soil-air interface in this pine forest was the result of complex interactions between the soil microbial community and both environmental and climatic variation. Much of the observed variation in CH4 flux was attributable to site location, soil moisture, and N treatment. However, a high level of replication in the experimental setup and a suite of environmental variables were needed to explain the full extent of variation in CH4 flux. The effects of location, N treatment and their interaction were also found to differ across time, but this did not appear to be a seasonal trend. This may be due to differing thresholds for the microbial response to N amendment in the two sites.

[32] There were many differences in soil texture and composition between the two neighboring sites, which are likely due to long-term disparities in soil moisture from elevation-driven drainage dissimilarities. There were significant differences in soil conditions and nutrient status, including bulk density, % N and % C, as well as inorganic nitrogen content, throughout the season. All soil measures showed greater differences between well and poorly drained sites, rather than between plots within each site. Therefore, in a deviation from much of the published literature, the differences in soil moisture alone did not drive methane flux variation in this forest, but rather the complex effects of how those differences in water table affected the soil in the long term.

[33] Soil moisture was the variable with the strongest correlation with CH4 flux, with higher CH4 release at greater soil moisture content. This is consistent with the published literature, since methanogens are known to be more active in anaerobic, poorly drained conditions, while methanotrophs need access to oxygen and CH4 in order to be active [Le Mer and Roger, 2001]. The correlation between soil moisture and CH4 consumption was significant, but low relative to those reported in the literature [Groffman and Pouyat, 2009; Adamsen and King, 1993]. A review of the literature found that at least 20 field CH4 flux studies showed some measure of soil moisture (gravimetric, by volume or by water filled pore space) to be the most important factor in determining CH4 flux (E. L. Aronson and B. R. Helliker, Review of non-wetland soil methane flux: Impacts of environment, climate change and methodology, submitted to Geoderma, 2011). Of those, 17 found increased moisture correlated with decreased CH4 consumption (or increased release). Another three studies found the opposite response. A study performed in desert soils [Angel and Conrad, 2009] and one in boreal forest [Ambus and Christensen, 1995] found that both CH4 release and consumption increased with dryer soils. The third study found both positive and negative relationships between water table height and CH4 release in boreal forest [van Huissteden et al., 2008]. Other studies found that soil moisture was relatively similar across sites and did not correlate with CH4 flux as well as it did with vegetation or other factors [i.e., Reay et al., 2005].

[34] The total magnitude of CH4 flux in the well-drained upper site was −5.38 kg CH4 ha−1 yr−1, if extrapolated across the year. This number is similar in magnitude to the global average for forest CH4 consumption reported by Dutaur and Verchot [2007] as −5.70 kg CH4 ha−1 yr−1, based on 92 temperate forest sites. The associated variance was 31.5 kg CH4 ha−1 yr−1, an order of magnitude larger than all other ecosystems they included in their analysis. Within temperate forests, Dutaur and Verchot [2007] showed coarse (sandy) soil CH4 flux to be −7.5, and organic soils −4.6, kg CH4 ha−1 yr−1, a range that brackets the flux in the upland site. Smith et al. [2000] found sites across Europe to range in average CH4 consumption rate from 0.1 to 9.1 kg CH4 ha−1 yr−1. That study found that the main determinant of whether soil was a net sink or source was the position of the surface relative to the water table, and that most soils were sinks [Smith et al., 2000]. In the Pine-lands, the poorly drained site represents a minority of the soil types and drainage regimes, so that the best estimate for overall flux comes from the well-drained site. If both the well- and poorly drained sites were weighted evenly, we would have found an average flux in the Pinelands of −0.84 kg CH4 ha−1 yr−1, which grossly underestimates the contribution of this site to the global CH4 sink. Many of the papers included in the review by Dutaur and Verchot [2007] and a meta-analysis [Aronson and Helliker, 2010] took snapshots of the ecosystems they studied in both space and time, not accounting for key environmental variation within these sites. Low levels of replication within some previously published studies of CH4 flux may be the reason their results are highly variable. When many of these results are taken together, the average flux across forests has a similar magnitude and level of variation to what we have found in the upland Pinelands forest in New Jersey.

[35] The control plots in the poorly drained, lower site did not release CH4 overall, and without N amendment the lower site had a net flux near zero. This is in contrast with most water-saturated soils, which are known to emit CH4 regularly. This finding hints that the patchiness of forests and the differences and environmental variations as the poorly drained site. Rather, the upper site consumed CH4 at a relatively
steady rate throughout the experimental timeframe. The CH$_4$ flux measurements in the lower site, however, were highly variable. This highlights the importance of soil moisture in determining CH$_4$ flux in both sites, rather than intermittent precipitation events, which did not correlate with changes in CH$_4$ flux. The well-drained site, likely due to its low water holding capacity and relatively low water table, may have shown resilience to perturbations of inorganic nitrogen content, precipitation, and temperature. Resilience to N disturbance was seen in the microbial community in a nearby, similar site by Krumins et al. [2009].

The interactive effects of site and N addition suggest that soil drainage conditions are important in predicting CH$_4$ responses to N levels. Upon first N application in the well-drained site, there was a nonsignificant effect of greater consumption with greater N addition that disappeared throughout the rest of the growing season. This may indicate that the microbial community in the well-drained soil is sensitive to N amendment, but undergoes rapid succession to a different, resistant community. Alternatively, the extant microbial community may have quickly adapted to the higher N condition. The first N applications did not impact CH$_4$ flux in the poorly drained site, but the high N treatment emitted more CH$_4$ after several applications. In the poorly drained site, therefore, the microbial community showed resistance to the perturbation of N amendment until a certain threshold is reached. At that point, either a well-adapted group of methanogens took over in the high N plots, or the N limitation of the existing methanogens was relieved; both explanations would result in the observed short burst of CH$_4$ release. The period of release may have ended because the methanotroph population grew in response and consumed the extra CH$_4$. There are multiple potential explanations for these observations, and in depth study of the microbial community may present findings that clarify which explanation is correct.

The rate of CH$_4$ release in the poorly drained site increased with high N addition, when averaged across the growing season. This result is consistent with the conclusions drawn in a meta-analysis on the same topic [Aronson and Helliker, 2010]. However, this trend was not seen in the well-drained site, probably due to the excessive drainage provided by the sandy texture of the upper site soil. There is an overlying organic layer of variable depth which may hold onto water and soluble compounds; the sand below, however, is known to drain excessively [Krumins et al., 2009], allowing rapid leaching. Soil texture is known to regulate CH$_4$ flux to a certain extent [Dutaur and Verchot, 2007], but there is little published information about the interactive effects of soil texture and moisture on CH$_4$ flux.

The data here presented are limited to net flux of CH$_4$ across the soil surface, and therefore trends cannot be attributed to variation in the community or actions of methanogens or methanotrophs specifically. As methanotrophs are thought to be more responsive to nitrogen variation than methanogens [Hanson and Hanson, 1996], it is possible that the increase in CH$_4$ release in poorly drained high N plots, was due to suppression of methanotrophy. However, it has been shown that under low N conditions, the addition of N fertilizer can stimulate methane consumption [Bodelier and Laanbroek, 2004], but also methanogenesis [Liu et al., 2011] so the origin of the observed responses is not clear. This is an active area of research and further study is necessary to identify clear causal relationships in CH$_4$ cycle responses to N addition [Bodelier, 2011].

Indirect effects may play a large role in the control of CH$_4$ flux across and between the two sites. N addition may have stimulated oxygen-consuming microbial activities in the poorly drained soil other than CH$_4$ oxidation. The resultant decrease in redox potential of the soil may have stimulated methanogenesis, driving the patterns seen in response to N addition in the lower site. Unfortunately, the oxygen sensors were not placed inside of treatment areas, and were only in place for half of the field season, so this effect was not tested directly. When we returned to the field site the following summer we did see a decrease in soil O$_2$ concentration in response to N addition, both in the lab and in the field. However, there was no significant associated change in CH$_4$ flux when studied in the growth chamber. Analysis of the microbial community itself is needed to approach these questions.

5. Conclusions

Although soil moisture was the greatest predictor of CH$_4$ flux across well- and poorly drained soils in this pine forest, it was necessary to create a complex statistical model using many environmental variables to capture the full variation in methane flux. The forest was a net sink for atmospheric CH$_4$ at rates that are similar to other forests across the globe. Drainage of the soil determined the response to environmental variation and treatments. The CH$_4$ flux rate in the well-drained soil showed either fast adaptation or resilience to perturbation, specifically N addition and natural variation in temperature and moisture. The poorly drained soil showed high variance that was driven by soil moisture and nitrogen addition, with a large release of CH$_4$ after a threshold of N addition was reached. These results are consistent with previous data on the inhibition of net CH$_4$ consumption by nitrogen addition. Further study of the microbial community should be performed to confirm the underlying cause of these trends in net CH$_4$ flux. The complex observed relationships between environmental variables and CH$_4$ flux indicate intricate relationships between environmental changes and microbial communities that deserve further study.

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