

## RESEARCH ARTICLE

# Soil Methane Sink Capacity Response to a Long-Term Wildfire Chronosequence in Northern Sweden

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## Abstract

Boreal forests occupy nearly one fifth of the terrestrial land surface and are recognised as globally important regulators of carbon (C) cycling and greenhouse gas emissions. Carbon sequestration processes in these forests include assimilation of CO<sub>2</sub> into biomass and subsequently into soil organic matter, and soil microbial oxidation of methane (CH<sub>4</sub>). In this study we explored how ecosystem retrogression, which drives vegetation change, regulates the important process of soil CH<sub>4</sub> oxidation in boreal forests. We measured soil CH<sub>4</sub> oxidation processes on a group of 30 forested islands in northern Sweden differing greatly in fire history, and collectively representing a retrogressive chronosequence, spanning 5000 years. Across these islands the build-up of soil organic matter was observed to increase with time since fire disturbance, with a significant correlation between greater humus depth and increased net soil CH<sub>4</sub> oxidation rates. We suggest that this increase in net CH<sub>4</sub> oxidation rates, in the absence of disturbance, results as deeper humus stores accumulate and provide niches for methanotrophs to thrive. By using this gradient we have discovered important regulatory controls on the stability of soil CH<sub>4</sub> oxidation processes that could not have not been explored through shorter-term experiments. Our findings indicate that in the absence of human interventions such as fire suppression, and with increased wildfire frequency, the globally important boreal CH<sub>4</sub> sink could be diminished.

## Introduction

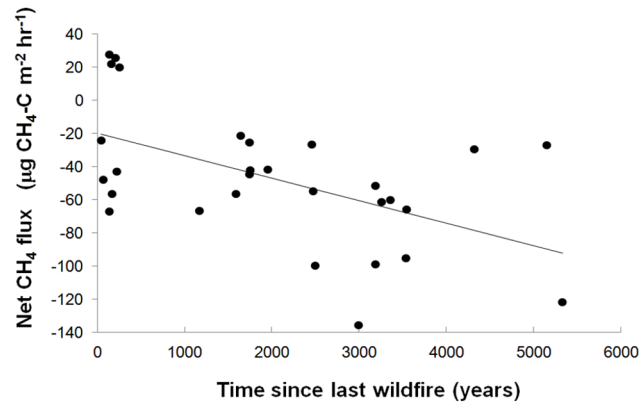
The Earth's boreal forests lie between 45° and 70°N and account for one third of all forested lands [1], covering between 9–12 million km<sup>2</sup>. As such, they play a major role in global

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carbon (C) storage and greenhouse gas regulation [2]. It is estimated that 37% of all terrestrial C is stored in boreal forests, which is greater than combined temperate and tropical stocks [1]. Currently, there is mounting concern for these high latitude C stores as they are predicted to undergo some of the strongest warming resulting from global climate change [3]. A possible consequence of rising temperatures is enhanced likelihood of wildfire ignitions and conflagration of forest biomass and organic soil by lightning strikes and human activity. It has been suggested that wildfire events could increase in frequency, intensity and extent as a result of a greater availability of drier fuel materials following more persistent and extreme drought events, and longer growing seasons due to earlier snow melt [4]. To date, both increases and decreases in fire events have been recorded, with diminishing fire frequency being associated with improved fire suppression techniques and habitat fragmentation as a result of land-use change [4]. This means that climate-driven increases in severe wildfire events may in part be balanced by human activity to suppress forest fires. Nonetheless, boreal forest wildfires burn between 3 and 23.6 million ha/year, representing an average of 13 million ha/year [5]. Wildfire events can have significant, large scale and prolonged impacts on terrestrial C storage as a consequence of both direct and indirect effects. In the short term, fire results in the conversion of forest biomass and soil organic C to CO<sub>2</sub> that is released to the atmosphere [6–7], whilst a smaller fraction of biomass enters soils as more recalcitrant black carbon [8]. Black carbon or charcoal additions can have varying effects on soil processes, at least for decades, including altering nutrient availability and increasing decomposition [9]. In the longer term, wildfires act as a fundamental regulator of succession, and thus C sequestration processes [2].

Globally, soils are the largest natural terrestrial CH<sub>4</sub> sink through biological oxidation by methanotrophic bacteria. It is widely accepted that in aerobic soils, methanotrophic activity [10] can be impaired for up to a century following soil disturbance events such as land use change or nitrogen addition [11]. To date, fire as a driver of this disturbance has received relatively little attention. At least in the short term (decadal), fire can increase [12–13], decrease [14] or have no change [15] for the net soil CH<sub>4</sub> sink, however, the underlying processes are poorly understood and are likely to be ecosystem dependent. Beneath forested organic rich wet soils recent fires have also been shown to alter soils from CH<sub>4</sub> sink to source [16]. Critically, however, the effects of increased or decreased wildfire frequency on soil CH<sub>4</sub> processes over longer (centennial or millennial) time scales is uncertain. The paucity of detailed studies of microbial CH<sub>4</sub> oxidation in boreal forest soils represents a serious gap in understanding that we sought to address in this study.

Here, we used a unique natural boreal forest fire chronosequence to determine the effects of historic fire disturbance on soil microbial methanogenesis and methanotrophy. This involved the study of 30 boreal forested islands located in freshwater lakes in Northern Sweden. These islands range in surface area from 0.02 to 15 ha. Lightning strikes were generally more frequent and thus more recent on larger islands; some large islands have burned as recently as 60 years ago while some small islands have not burned for 5,000 years [17–20]. Consistent with previous work across this island gradient, we have classified these islands as early (time since last fire (mean ± SE) = 585 ± 233 years; area > 10 ha; n = 10), mid (2,180 ± 385 years; area of 0.1 to 1.0 ha; n = 10) and late (3,250 ± 439 years; area < 0.1 ha; n = 10) successional stages [17–19]. Using this natural fire chronosequence and substituting space for time, we aimed to better understand how ecosystem retrogression, as driven by wildfires, affects the soil-atmosphere exchange of CH<sub>4</sub>. We made a series of in-situ and ex-situ measurements of soil CH<sub>4</sub> fluxes to characterise net CH<sub>4</sub> fluxes during two successive growing seasons across the gradient of 30 islands.

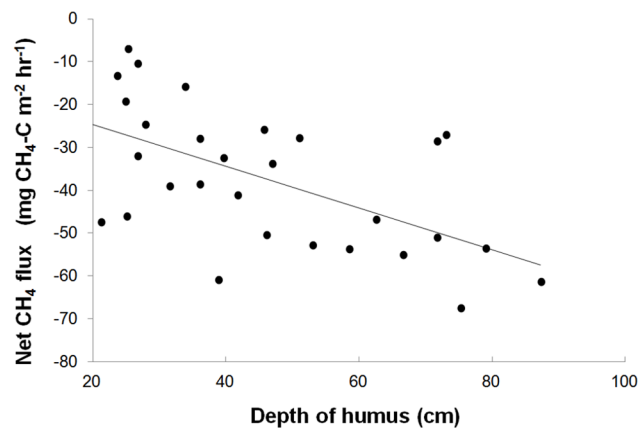


**Fig 1. In situ CH<sub>4</sub> flux versus time since last fire.** *In situ* 2006 CH<sub>4</sub> chamber flux data versus time since last fire ( $R^2 = 0.4$ ;  $P = 0.001$ ). Each data point represents the mean of five measurements made on each island at the same time. 10 depth measurements were made per island.

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## Results and Discussion

The effect of time elapsed since last wildfire event on *in situ* net soil CH<sub>4</sub> fluxes was examined using a static chamber method ( $n = 5$  per island, 30 islands). Results show that the net soil CH<sub>4</sub> sink significantly increases ( $R^2 = 0.40$ ,  $P < 0.001$ ) with time since last wildfire disturbance (Fig 1). We propose that this effect is mediated by successional changes in the plant community since fire disturbance. As time since fire increases, vegetation community composition shifts from domination by early successional *Pinus sylvestris* and *Vaccinium myrtillus*, to the late successional *Picea abies* and *Empetrum hermaphroditum* [18, 20]. The late succession plant community is characterised by having recalcitrant litter inputs, impaired productivity and decomposer activity, and greater humus depth and C storage (S1 Table) [17–20]. The build-up of soil organic matter was observed to increase significantly with time since fire disturbance ( $R^2 = 0.41$ ;  $P \leq 0.001$ ), with a significant correlation between greater humus depth and net soil CH<sub>4</sub> oxidation rates ( $R^2 = 0.35$ ;  $P \leq 0.001$ ; Fig 2). Soil moisture variation in the top 25 cm of humus, a common driver of short-term soil CH<sub>4</sub> oxidation rates, was found to have a weak but positive correlation with the net CH<sub>4</sub> oxidation rates ( $R^2 = 0.15$ ;  $P = 0.03$ , Table 1), whilst no



**Fig 2. In situ CH<sub>4</sub> flux versus depth of humus.** *In situ* 2006 CH<sub>4</sub> flux versus depth of humus ( $R^2 = 0.35$ ;  $P \leq 0.001$ ). Each data point represents the mean of five measurements made on each island at the same time. 10 depth measurements were made per island.

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**Table 1. Soil moisture determinations (%) made from soil cores from early, mid and late succession islands.**

Year	Depth (cm)	Early	Mid	Late	F	P
2006	0–25	54.2 <sup>a</sup> (3.7)	58.8 <sup>a,b</sup> (2.2)	66.6 <sup>b</sup> (1.7)	5.42	0.01
2007	0–20	71.3 <sup>a</sup> (4.8)	73.3 <sup>a</sup> (3.2)	74.5 <sup>a</sup> (0.9)	0.23	0.8
2007	20–40	66.9 <sup>a</sup> (13.3)	81.6 <sup>b</sup> (1.5)	76.8 <sup>ab</sup> (0.6)	3.45	0.05
2007	40–60	nd	78.3 <sup>a</sup> (0.8)	79.3 <sup>a</sup> (0.9)	0.39	0.55
2007	60–80	nd	nd	82.0 (2.7)	nd	nd

For 2006 all values in brackets are standard errors of the mean (n = 10). Values followed by the same letter in superscript indicate no significant difference at P < 0.05, d.f = 2,27. For 2007, 20–40 cm values in brackets are standard errors of the mean for early (n = 3), mid (n = 8) and late (n = 10), d.f = 2,18; 40–60 cm values in brackets are standard errors of the mean for mid (n = 3) and late (n = 8), d.f = 1,9; 60–80 cm values in brackets are standard errors of the mean for late (n = 5). 'nd' indicates where samples could not be obtained due to a shallow humus depth.

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relationship was identified with bulk density. Using island area classes that corresponded with plant successional classifications, early successional islands showed significantly lower net CH<sub>4</sub> oxidation than islands at mid or late successional stages (Table 2). Overall, these results offer strong evidence that deeper soils of later post-fire successional forest stages provided conditions for enhanced methanotrophic activity.

We targeted CH<sub>4</sub> flux measurements on the uppermost 25 cm of the soil profile where previous studies have shown evidence of maximal methanotrophic activity [21–22]. Intact 25 cm deep soil cores (n = 4 per island x 30) were sampled from each island. These cores were then examined in *ex situ* laboratory incubations where we did not detect a relationship between net CH<sub>4</sub> fluxes and time since last fire, or with successional stage (Table 2). This result, combined with net field CH<sub>4</sub> flux data, suggests that significant CH<sub>4</sub> oxidation must be occurring at depths greater than 25 cm. To investigate this we returned to the islands in of the following year and collected whole humus soil profile cores (up to 85 cm depth to bedrock) from each island. These were taken back to the laboratory to carry out CH<sub>4</sub> flux measurements under controlled environmental conditions. Another possible cause for the lack of observed differences in CH<sub>4</sub> oxidation rates from the 25 cm cores was our *ex-situ* methodologies; *ex-situ* approaches by their nature are disruptive, for example, through altering diffusional rates of CH<sub>4</sub> from the atmosphere or by removing the supply of CH<sub>4</sub> from deeper anaerobic regions. By measuring CH<sub>4</sub> fluxes from deeper cores we aimed to confirm that this was not the case. During the second year's campaign soils were considerably wetter than in the previous (Table 1). The observed rates of *ex situ* CH<sub>4</sub> oxidation from the deep cores was lower than the previous *in situ* and *ex situ* measurements made during the previous year, however, net CH<sub>4</sub> oxidation rates were in the order Late > Mid > Early successional islands (Table 2). Thus, comparing net CH<sub>4</sub> oxidation results from our 25 cm and the deep cores indicate that

**Table 2. Net CH<sub>4</sub> oxidation rate of early, mid and late succession islands for *in situ* and *ex situ* soil core measurements.**

Measurement	Early	Mid	Late	F	P
<i>In situ</i> 2006	-25.76 <sup>a</sup> (4.28)	-40.32 <sup>b</sup> (4.61)	-45.20 <sup>b</sup> (4.88)	4.84	0.02
<i>Ex situ</i> 2006 (25 cm core)	-11.08 <sup>a</sup> (2.31)	-12.81 <sup>a</sup> (2.50)	-9.09 <sup>a</sup> (2.82)	0.53	0.59
<i>Ex situ</i> 2007 (up to 85 cm core)	-6.38 <sup>a</sup> (2.55)	-11.99 <sup>a,b</sup> (2.44)	-16.52 <sup>b</sup> (2.90)	3.70	0.04

Values in brackets are standard errors of the mean (n = 10). Values followed by the same letter in superscript indicate no significant difference at P < 0.05, d.f = 2,27.

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methanotrophic activity is prevalent in deeper soil layers that form under the prolonged absence of wildfire.

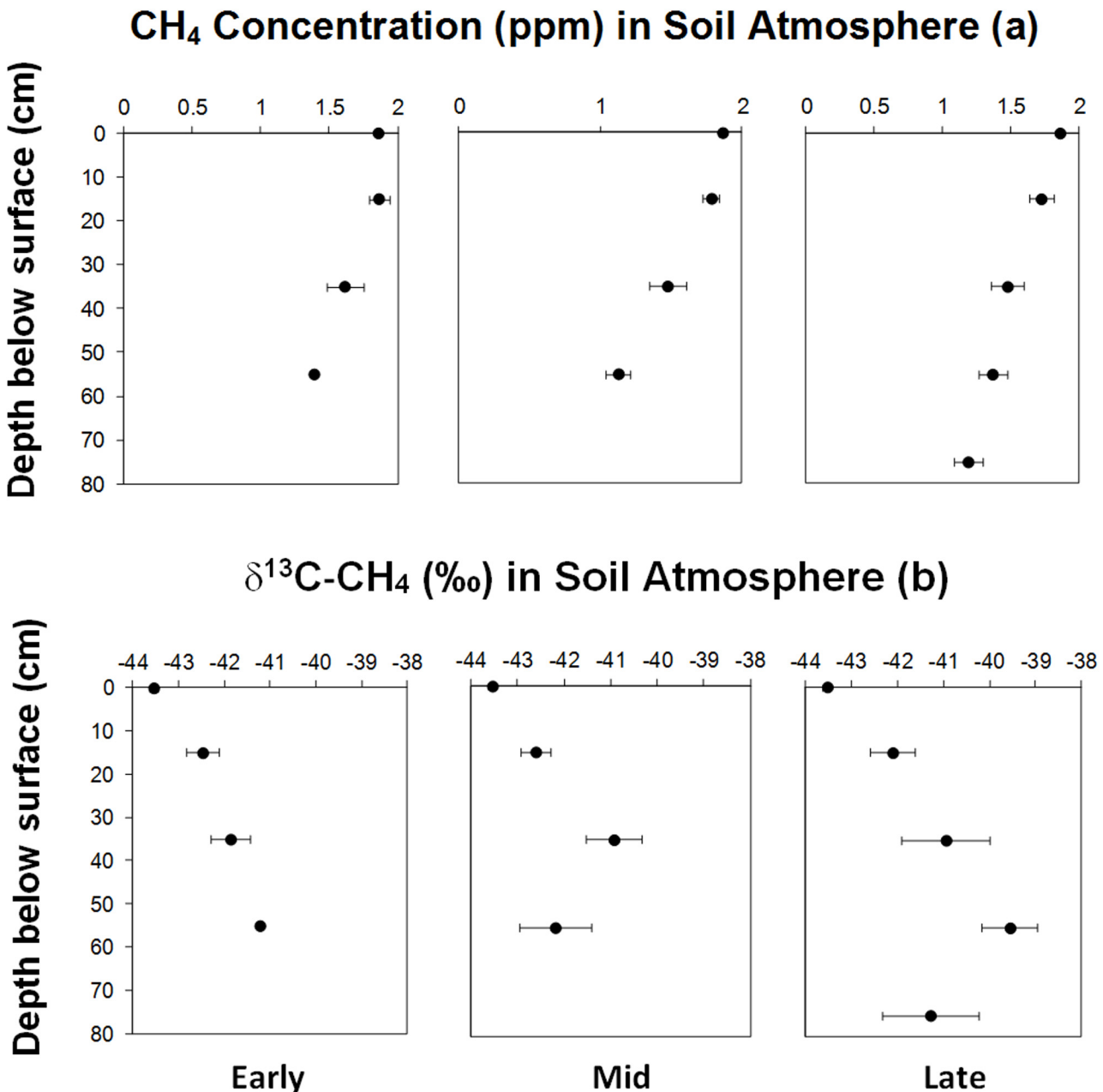
Based on our *in-situ* results from our first campaign we hypothesised that methanogenic processes were concurrent in deeper humus layers, thus potentially priming methanotrophic activity through provision of additional CH<sub>4</sub> substrate supply [23]. During our second field campaign we therefore used a natural abundance stable isotope *in situ* approach to gain additional insight into CH<sub>4</sub> processes at depth. For this, a series of sub-surface CH<sub>4</sub> concentration measurements were made across the islands at a range of soil depths. These measurements allowed us to initially determine whether we could detect above ambient concentrations of CH<sub>4</sub> as evidence of active methanogenesis in the soil profile. Concentration of CH<sub>4</sub> measured in the humus profile were consistently below ambient levels, suggesting that either methanogenesis was not dominant in these soils during the peak growth season (Fig 3A), or that rapid oxidation of CH<sub>4</sub> by the methanotroph community was removing any endogenous CH<sub>4</sub> production. Analyses of the stable <sup>13/12</sup>C isotope ratio of CH<sub>4</sub> in these samples confirmed that methanogenesis was not occurring, as δ<sup>13</sup>C values of <sup>13</sup>CH<sub>4</sub> were enriched relative to atmospheric CH<sub>4</sub> (Fig 3B). It has been shown that methanotrophs preferentially assimilate lighter isotopes, leaving residual CH<sub>4</sub> that is <sup>13</sup>C enriched [24]. Our results demonstrated that both CH<sub>4</sub> concentrations and the δ<sup>13</sup>C values of <sup>13</sup>CH<sub>4</sub> were strongly influenced by the depth of the humus profile (Table 2). Using these data, we quantified the CH<sub>4</sub> oxidation rate at sub-surface depths *in situ* using a ‘top-to-bottom difference’ <sup>13</sup>C-CH<sub>4</sub> modelling approach [24–25]. We found that soils typically oxidised between 2–5% of CH<sub>4</sub> in the atmosphere (Table 3), with deeper soils leading to a higher cumulative CH<sub>4</sub> oxidation rate (Table 4). As these soils were not found to produce CH<sub>4</sub>, the supply of this substrate to deeper soil profile was from the atmosphere. This increasing CH<sub>4</sub> oxidation at depth corresponded to the sub-surface minimum CH<sub>4</sub> concentrations detected in the humus profile recorded with our *in-situ* depth measurements (Fig 3A), and also in *ex situ* profile cores (up to 85 cm) taken during the second field campaign (S2 and S3 Tables). Collectively, these results support the notion that increasing time since wildfire has indirectly affected the CH<sub>4</sub> oxidation process by enabling the development of deeper humus profiles which harbour the niches necessary for methanotrophs.

Our study reveals, for the first time, that across contrasting islands differing in exposure times to historical fire disturbance, net CH<sub>4</sub> oxidation processes vary predictably. Specifically, we have shown that low productivity, old growth boreal forest ecosystems that have not burned for up to 5000 years develop deeper humus layers, leading to enhanced soil microbial CH<sub>4</sub> oxidation. Crucially, our results indicate that disturbance of these humus C stores will threaten the stable microhabitat conditions required for methanotrophs to thrive. In the future, climate change is expected to result in increased wildfire ignitions from lightning and increased fire season length [26]. These same drivers are also expected to increase wildfire severity which will in turn promote soil organic matter loss and methanotrophic community disturbance. Together, these global changes could reduce the apparent natural CH<sub>4</sub> sink capacity of late succession boreal forest soils, with potential feedback to greenhouse gas forced climate warming. Our results indicate that in the absence of human interventions, such as fire suppression, the globally important boreal CH<sub>4</sub> sink could be diminished by future wildfire events.

## Materials and Methods

### Ethics Statement

This study was made on 30 freshwater islands in the Boreal forest region of northern Sweden. These islands are part of an archipelago of over 400 islands within the lakes Uddjaure and Hornaven (N65° 15' N; 17° 43' to 66° 99' N; 17° 55' E). These islands are not within national



**Fig 3. Mean *in situ* concentration depth profiles of CH<sub>4</sub> (a) and distribution of δ<sup>13</sup>C-CH<sub>4</sub> (b) in boreal forest soils.** The depth at surface (0 cm) represents the ambient concentration and δ<sup>13</sup>C-CH<sub>4</sub> value. Error bars represent ± standard error (n = 10). The changes in CH<sub>4</sub> and CO<sub>2</sub> concentration and δ<sup>13</sup>C-CH<sub>4</sub> with depth were only significant in mid and late successional stages (P ≤ 0.05). Statistical analyses of data are shown in [Table 2](#).

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reserves, and the land is public and not government protected. We confirm that all national and international rules were complied with during the field work and no specific permissions were required to access the islands. The research did not involve measurements on humans or animals. The soil and plant material collected for this study had negligible effects on broader ecosystem functioning. We have no commercial interests or conflicts of interest in performing this work.

**Table 3. Percentage of atmospheric CH<sub>4</sub> oxidised in the soil profile and statistical analysis of whole profile % CH<sub>4</sub> oxidised for early, mid and late succession islands.**

Successional Stage	CH <sub>4</sub> (%) oxidised at specific depth ranges								Whole profile	n
	0–15 cm	n	0–35 cm	n	0–55 cm	n	0–75 cm	n		
Early	1.47 (0.5)	10	2.30 (0.61)	6	3.18 (NA)	1	nd	0	2.02 (0.45) <sup>a</sup>	10
Mid	1.27 (0.45)	10	3.66 (0.85)	9	1.88 (0.9)	6	nd	0	3.1 (0.83) <sup>a</sup>	10
Late	1.96 (0.68)	10	3.73 (1.41)	10	5.63 (0.80)	9	3.14 (1.51)	4	5.09 (1.15) <sup>a</sup>	10

Model assumes transport of CH<sub>4</sub> in to the soil by diffusion ( $\alpha_{transport} = 1.0195$ ). The whole profile estimate considers the total CH<sub>4</sub> oxidised for the given depths of humus. Values in brackets are standard errors of the mean. For early and mid islands 'nd' indicates where samples could not be obtained due to a shallow humus depth. The number of depths sampled is given by n. For whole profile analyses values followed by the same letter in subscript indicate no significant difference at  $P < 0.05$ ,  $F = 2.6129$ ,  $P = 0.09176$ ,  $d.f = 2, 27$ .

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### Study System

The lakes were formed when the retreat of land ice 9,000 years ago left behind granite and moraine boulders to form the foundation of the current islands. All of these islands are of the same geological age and experience the same climatic conditions, having a mean annual precipitation of 750 mm and mean temperature of +13°C in July and -14°C in January. All islands are the same distance from the shore irrespective of island size which prevents confounding effects of erosion, wind and radiation. The forested portions of the islands are not subjected to other major disturbances such as flooding and ice formation, as the level of the lake is always below the level of the lowest forested vegetation. The only extrinsic factor which varies among the islands is wildfire frequency [17–19]. The 30 islands are of varying size (0.02 to 15 ha) with lightning strikes being more frequent on larger islands as they have a larger area to intercept these strikes. The age of major wildfire events was previously determined using <sup>14</sup>C dating of buried charcoal fragments and through examination of fire scars from the last 250 years [17–20]. These islands collectively form a retrogressive chronosequence, with retrogression increasing with decreasing island size. A description of island properties is given in [S1 Table](#).

**Table 4. Statistical analyses for the effects of, and interactions between, depth and successional stage on *in situ* profile CH<sub>4</sub> concentration,  $\delta^{13}C-CH_4$  and % CH<sub>4</sub> oxidised.**

<b>CH<sub>4</sub> concentration</b>	F	P
Stage (S)	3.2975	0.0529
Sample Depth (SD)	53.4777	0.0001
Stage x Sample Depth (S x SD)	2.5726	0.0883
<b><math>\delta^{13}C-CH_4</math></b>	F	P
Stage	2.061	0.1469
Sample Depth	8.336	0.0061
Stage x Sample Depth	0.150	0.8614
<b>% CH<sub>4</sub> oxidised</b>	F	P
Stage	2.13987	0.1372
Sample Depth	8.03978	0.0070
Stage x Sample Depth	0.15000	0.8602

df: S = 2, 26, SD = 1, 42, S x SD = 2, 42

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## Determining *in situ* CH<sub>4</sub> fluxes

The *in situ* gas fluxes were measured over one week using a static chamber method during August 2006 ( $n = 5$  per island; chamber volume =  $0.0054 \text{ m}^3$ , diameter =  $0.2 \text{ m}$ ). Cylindrical chamber base rings were installed and subsequently removed on the day of measurement. The installation involved gently cutting the base ring into the humus to  $5 \text{ cm}$  depth. Headspace gas samples,  $9 \text{ ml}$ , were drawn at  $0, 5, 10, 15, 20, 25$  and  $30$  minutes after an opaque chamber lid was sealed onto the base ring. Samples were stored in exetainer vials (Labco, UK) for up to one month prior to analyses. Concentrations of CH<sub>4</sub> were analysed by gas chromatography, on a PerkinElmer Autosystem XL Gas Chromatograph (GC) with Flame Ionization Detector, and results were calibrated using certified gas standards. CH<sub>4</sub> was separated isothermally on a  $2 \text{ m}$  Poropak Q 50–80 mesh packed column at  $40^\circ\text{C}$ , with N<sub>2</sub> as the carrier gas. The mean detectable concentration change was  $0.05 \text{ ppm CH}_4$  which was equivalent to  $3 \mu\text{g CH}_4 \text{ m}^{-2} \text{ hr}^{-1}$  [27]. Where fluxes fell below this detection limit they were still included in the analyses following others [27–29]. Calculations of the rates of exchange of CH<sub>4</sub> are based on a standard linear regression methodology [30].

## Determining *ex situ* CH<sub>4</sub> fluxes from intact cores

We measured CH<sub>4</sub> fluxes from *ex situ* cores from the top  $25 \text{ cm}$  of soil (or to the bedrock where soil depth was less than  $25 \text{ cm}$ ) in 2006 and then through the entire humus profile to the bedrock ( $20$ – $85 \text{ cm}$  depth) in 2007. In August 2006, four intact cores ( $11 \text{ cm}$  diameter,  $25 \text{ cm}$  depth) were removed from each island ( $40$  per island size classification) and were weighed on the day of collection. Cores were stored at  $12^\circ\text{C}$  in the dark until measurement and were maintained at their field collected moisture content using an artificial rain solution. The artificial rain solution was made up according to a formula which results in rainwater with a pH of  $5.6$  with a conductivity of  $22.4 \mu\text{S}$  (S4 Table). Cores were grouped into late, mid and early successional stage dependent upon the island from which they were removed ( $n = 10$ ). Soil CH<sub>4</sub> fluxes were measured using the static chamber method (volume =  $0.0008 \text{ m}^3$ ) with sampling at  $0, 30, 60, 90, 120$  and  $150$  minutes after headspace closure. In 2007, one full profile core ( $0.15 \text{ m}$  diameter) was collected from each island humus profile to the bedrock in July 2007. A similar static chamber approach (volume =  $0.0021 \text{ m}^3$ , diameter =  $0.15 \text{ m}$ ) for measuring soil CH<sub>4</sub> fluxes, applied under the same measurement conditions as previously described. Sample storage using  $3.6 \text{ ml}$  exetainers vials, GC analysis and data analysis was as described for the *in situ* CH<sub>4</sub> sample analyses. Soil moisture was calculated after drying soils in an oven at  $105^\circ\text{C}$  until no further moisture loss was recorded.

## *In situ* depth profiles of $\delta^{13}\text{C}_{\text{CH}_4}$ and concentrations of CH<sub>4</sub>, and CO<sub>2</sub>

*In situ* gas samples were collected from within the soil atmosphere in the humus profiles of each island over 4 days in July 2007. Four stainless steel sampling probes ( $19$  gauge,  $0.69 \text{ mm}$  inner diameter) were inserted into the soil profiles  $30$  minutes before soil atmosphere samples were collected at  $15, 35, 55$  and  $75 \text{ cm}$  depth (dead-space volume =  $0.06, 0.13, 0.21, 0.28 \text{ ml}$ , respectively). From each probe a  $100 \text{ ml}$  sample was collected with  $90 \text{ ml}$  being transferred to a  $50 \text{ ml}$  Wheaton bottle with butyl rubber stopper and crimp top seals and  $9 \text{ ml}$  to a  $3.6 \text{ ml}$  exetainer vial. After collection each butyl rubber stopper and crimp top was sealed with wax. Analyses of the  $\delta^{13}\text{C}$  values of CH<sub>4</sub> were completed within one month at the Natural Environmental Research Council Life Sciences Mass Spectrometry Facility at the Centre for Ecology & Hydrology, Lancaster, using an Isoprime Ltd Tracegas Preconcentrator coupled to an Isoprime Ltd Isoprime isotope ratio mass spectrometer. Samples were initially passed through a Magnesium perchlorate/Carbosorb scrubber trap at  $20 \text{ ml min}^{-1}$  to eliminate water



and CO<sub>2</sub>. The CH<sub>4</sub> was then oxidized in a combustion furnace using a braided platinum/copper/nichrome furnace wire inside a ceramic furnace tube of 200 mm x 0.4 mm i.d. heated in a furnace at 950°C. A preparation flow rate of 10 psi was required to give a flow rate of 20 ml min<sup>-1</sup> through the furnace at full operating temperature. Precisions were better than or equal to 0.2 ‰. The δ<sup>13</sup>C of CH<sub>4</sub> was expressed in per mil and calculated with respect to Pee Dee Belemnite (PDB). Reference CO<sub>2</sub> was calibrated to National Institute of Standard Technology (NIST) certified reference materials (N.I.S.T. 8559 and 8561) and working CH<sub>4</sub> standards (Scientific and Technical Gases Ltd).

### Isotopic method to determine the quantity of CH<sub>4</sub> oxidised

Non-equilibrium or kinetic isotope effects (KIEs) associated with methanotrophy in soils are denoted using the isotope fractionation factor ( $\alpha_{ox}$ ). The KIE represents the different rates of uptake of the CH<sub>4</sub> isotopologues (<sup>12</sup>CH<sub>4</sub>, <sup>13</sup>CH<sub>4</sub>), with  $\alpha_{ox}$  being the ratio of the rate constants of isotopically light and heavy CH<sub>4</sub> for both physical and biological processes associated with methanotrophy in soil [24–25]. The resultant isotopic fractionation factor for CH<sub>4</sub> oxidation ( $\alpha_{ox}$ ) is known to vary across boreal soils [24], therefore it was necessary to determine  $\alpha_{ox}$  for each island. A straightforward and effective method of determining  $\alpha_{ox}$  is by using the ‘Top-to-Bottom’ δ<sup>13</sup>C-CH<sub>4</sub> approach [24–25]. This allows the determination of the fraction of atmospheric CH<sub>4</sub> as it passes through soil layers. It involves calculating the difference in δ<sup>13</sup>C-CH<sub>4</sub> values from atmospheric CH<sub>4</sub> samples and samples taken from the soil atmosphere at depth. This difference is then added to 19.7 ‰ to take account of fractionation by gas diffusion through the soil due to the higher translational velocity of <sup>12</sup>CH<sub>4</sub> versus <sup>13</sup>CH<sub>4</sub> in air filled pores spaces. Diffusion of CH<sub>4</sub> into the soil tends to result in isotopic depletion as <sup>12</sup>CH<sub>4</sub> diffuses more quickly along its gradient than <sup>13</sup>CH<sub>4</sub> causing <sup>12</sup>CH<sub>4</sub> to travel further in soil pore space. During the process of CH<sub>4</sub> oxidation the remaining CH<sub>4</sub> becomes heavier as <sup>12</sup>CH<sub>4</sub> is more readily oxidised by methanotrophs. The calculated isotope fractionation factors ( $\alpha_{ox}$ ) for this work are given in S4 Table.

To determine the fraction of CH<sub>4</sub> oxidised by boreal forest soils, a mass balance model was used that accounted for the fractionation effects of both biological oxidation and transport of CH<sub>4</sub> from the atmosphere. The model is adapted from previous work that estimated CH<sub>4</sub> oxidation in landfill [31] cover soils, but has since been used in natural systems to investigate CH<sub>4</sub> cycling in humid tropical forests [32]. Eq 1 describes the model:

$$F_o = (\delta_{soil} - \delta_{atmos}) / [(\alpha_{ox} - \alpha_{trans}) \times 1000] \quad \text{Eq1}$$

where  $F_o$  represents the fraction of CH<sub>4</sub> oxidised. To calculate the percentage of CH<sub>4</sub> oxidised,  $F_o$  is multiplied by 100.  $\delta_{soil}$  and  $\delta_{atmos}$  represent the isotopic composition (‰) of CH<sub>4</sub> at soil depths and atmosphere respectively.  $\alpha_{trans}$  is the isotopic fractionation associated with gas transport. There is no method to directly calculate  $\alpha_{trans}$ . Other studies have assumed movement of CH<sub>4</sub> by advective transport [31] which involves no isotopic fractionation of CH<sub>4</sub> giving a  $\alpha_{trans}$  value of 1. Using this value can result in an underestimation of CH<sub>4</sub> oxidation. As in this study, it was not possible to calculate  $\alpha_{trans}$ ,  $F_o$  was calculated using  $\alpha_{trans}$  values of 1.0195, which is the diffusion fractionation factor reported for temperate forest soils [33].

### Ecosystem properties

Using our 2006 collected soil cores, we measured soil pH, % carbon and % nitrogen. Soil pH was determined in a soil:water ratio of 1:3 using the Hanna pH 210 Microprocessor pH Meter. The percentage of soil C and N was analysed by oxidative combustion, followed by thermal conductivity detection using an Elementar Vario EL analyser (Elementar Analysensysteme

GmbH, Hanau, Germany). In July 2007 the humus depth of each island was assessed by taking ten depth measurements along a transect across approximately the centre of each island. Depths were sampled at equal intervals by inserting a metal rod to the bedrock. Additional existing information on vegetation mass, shrub productivity, tree productivity and humus mass are as reported previously [17–20].

## Statistical Analyses

All analyses were conducted with the R statistical program (R Development Core Team, 2011). Simple regression analyses were used to model the relationship between response variables and predictors (successional age of system from  $^{14}\text{C}$  dating, humus development). Differences in island soil moisture content, *in situ* and *ex situ*  $\text{CH}_4$  fluxes between successional stages (Early, Mid and Late) were tested with the *glm* function. This same approach was applied when testing for differences between the quantity of  $\text{CH}_4$  oxidised for successional stages using the stable isotope approach. A constant value, equal to lowest datapoint + 1, was added to each datapoint to ensure all data were non-negative and a quasi-likelihood function with a log link and constant variance parameter used to account for the non-normal distribution. Differences in other  $\text{CH}_4$  measures ( $\text{CH}_4$  concentration,  $\delta^{13}\text{C}$  of  $\text{CH}_4$  and %  $\text{CH}_4$  oxidised) across successional stages and soil depths were tested with a linear mixed effect model using the *lme* function in the *nlme* package. Island was included as a random term to account for dependencies between measures made on the same core. Diagnostic plots of residuals versus fitted values were inspected to ensure there was no bias or heteroscedasticity in model residuals.

## Supporting Information

**S1 Table. Changes in island properties for each successional class across the island size gradient.**

(DOCX)

**S2 Table. Mean  $\text{CH}_4$  concentration (ppm) in soil air of *ex situ* cores at stratified depths.**

(DOCX)

**S3 Table. Statistical analyses for the effects of, and interactions between, depth and successional stage on *ex situ* profile  $\text{CH}_4$  concentrations.**

(DOCX)

**S4 Table. Kinetic Isotope Effect (KIE) for soil methanotrophy on early, mid and late succession islands.**

(DOCX)

**S5 Table. Artificial Rain Solution.**

(DOCX)

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## Author Contributions

Conceived and designed the experiments: NPM RG JCM DAW RDB NJO. Performed the experiments: NPM RG AS SO MdTR. Analyzed the data: NPM DAW NJO. Contributed

reagents/materials/analysis tools: NPM NJO JCM AS. Wrote the paper: NPM JCM MdTR DAW RDB NJO.

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