Swedish University of Agricultural Sciences
Department of Soil and Environment

Methane Oxidation in three Swedish Forest Soils

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ABSTRACT

Aerated soils are the largest sinks of atmospheric methane on the Earth's surface. Methane removal from the atmosphere is performed through oxidation by microorganisms (methane-oxidising bacteria) in the soil. Methanotrophic bacteria utilise methane (single-carbon compound) as their main carbon and energy source, but some groups have been found to grow on different multi-carbon compounds. This thesis investigated the influence of soil methanotrophs on the kinetics of methane oxidation in Swedish forest soils and the effect of the tree species Scots pine *(Pinus sylvestris)*, Norway spruce *(Picea abies)* and birch *(Betula pendula)* on methane consumption rates. Soil samples were collected from the study area Vipängen (Ultuna), which is classified as a Dystrocryept with its O horizon between 0-5 cm, A horizon 5-20 cm and B horizon 20-40 cm. The potential effect of other multi-carbon substrates (acetate, vanillic acid and guaiacol) that could be expected to enhance the oxidation rate of atmospheric methane was also examined. The results showed that the methanotrophs at the study site were high-affinity species with V_{max} values ranging between 4.3 and 11.0 nmol CH_4 g^{-1}d.w. hr^{-1} and K_m ranging between 7.4 and 185.5 nmol g^{-1}d.w. Type of tree species which is proposed to have a strong influence on the methane sink, clearly did so in this incubation experiment. Consumption in soil samples from a birch stand displayed the highest consumption rate, followed by spruce and pine for all four different initial concentrations of methane tested (4.5, 7, 14.1 and 45.1 ppmv). Evaluation of the effects of acetate, vanillic acid and guaiacol on methane consumption rates in the Swedish forest soils studied showed that only addition of acetate yielded a substantial effect, demonstrating that methanotrophs are not just limited to single-carbon bonds. Further studies on the effects of different multi-carbon compounds on the growth of some methanotrophic species (increased oxidation) will provide in-depth knowledge of the factors governing methane fluxes between the atmosphere and the soil.

CONTENTS

1. INTRODUCTION AND BACKGROUND

Methane is second to carbon dioxide in terms of abundance of atmospheric long-lived greenhouse gases. It contributes about 20 % of the observed global warming reported by the Intergovernmental Panel on Climate Change (IPCC, 2007). Methane is a fairly reactive trace gas, with an atmospheric lifespan of approximately 7.9 years.

Records of atmospheric methane concentrations for the period 1750 to 2007 showed an increase from 0.71 ppm to 1.77 ppm (IPCC, 2007), with a current concentration of about 2 ppm. Methane represents the most abundant organic compound present in the Earth's atmosphere (Lelieveld et al., 1998).

Estimates of global atmospheric methane budget are accredited to two main categories of natural and anthropogenic sources and two main removal processes (IPCC, 2007). The major natural source of atmospheric methane is attributed to emissions from wetlands (Cai et al., 2001), while the main anthropogenic source is emissions from fossil fuel combustion. The major removal process is the stratospheric reaction with free hydroxyl radicals and the biospheric reaction of oxidation by microorganisms in soils ($CH_4+2O_2 \rightarrow CO_2+2H_2O$; $\Delta G°=-818$ kJ mol^{-1}). An illustration of the methane conversion processes is given in Figure 1.

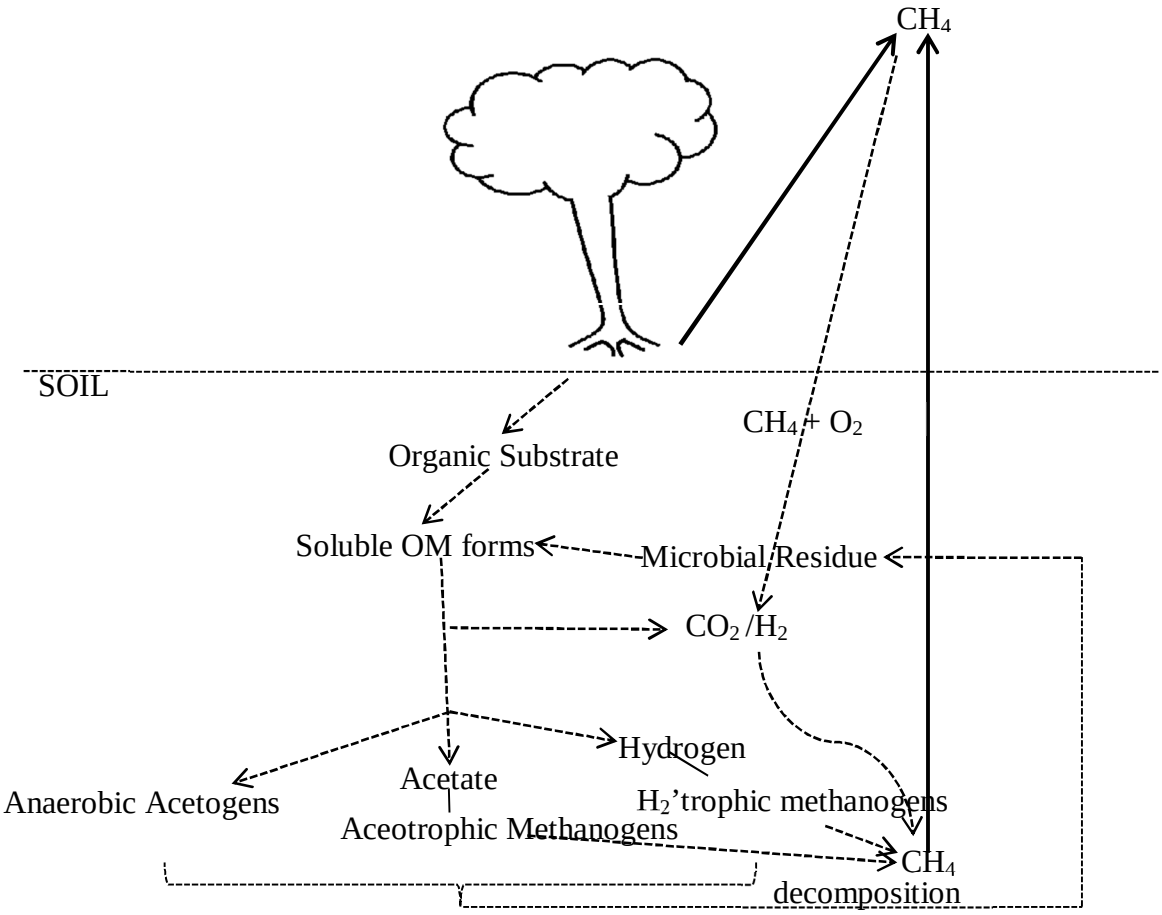

Figure 1: Pathway of methane production and consumption in the soil environment and its emission to the atmosphere (adapted from Dalal and Allen 2008).

1.1. Forest soils as sinks of atmospheric methane

Aerated soils serve as sinks for atmospheric methane, with forest soils playing a significant role. Temperate forest soils are reported to have the highest consumption rate of atmospheric methane at -4.8±0.6 kg CH_4 $ha^{-1}y^{-1}$ as compared to other forest soil types (Dalal and Allen, 2008). Forest cover type and stand species composition have been suggested to strongly alter methane oxidation rates (Borken et al., 2003; Menyailo and Hungate, 2003; Reay et al., 2005; Borken and Beese, 2006), and also influence some regulating soil environmental factors such as soil moisture content and soil pH (Angers and Caron, 1998; Hooper et al., 2000). Although these factors contribute to the overall sink strengths, the various processes underlying these factors are not well understood.

Another important factor for methane oxidation in forest soils is differences in soil chemical composition, such as the presence of monoterpenes generated by different tree species. Monoterpenes are naturally occurring volatile organic compounds which constitute a major part of the plant's essential oils (Amaral and Knowles, 1998). The monoterpenes are derivatives of isoprene ($C_{10}H_{12}$) and may either be linear or cyclic (Connolly and Hill, 1991). A variety of monoterpene is thought to inhibit growth and activity of methanotrophic bacteria, thereby inhibiting the oxidation rate of methane in forest soils (Amaral and Knowles, 1997).

1.2. Methane-oxidising bacteria

Methane-oxidising bacteria (methanotrophs) as well as nitrifiers are responsible for the oxidation processes of atmospheric methane within forest soils. Methanotrophs are distinct groups of prokaryotes being able to utilise and metabolise methane as their sole carbon and energy source. They are able to grow in aerobic environments and require single-carbon bond compounds for their survival (Lidstrom, 2006). Methanotrophs oxidise methane into carbon dioxide through conversion of the gas into methanol and formaldehyde, which are used in cellular processes for biomass production, and the excess carbon from energy production is converted into carbon dioxide. The first step in the oxidation pathway is catalysed by a unique type of enzyme, methane monooxygenase (MMO), which depending on the type of species occurs either as soluble in the cytosol (sMMO) or as particulate membrane-bound (pMMO) (Bowman et al., 2006).

Methanotrophs are divided into two main groups, Type I and Type II, belonging to the γ- and α- proteobacteria, respectively. This grouping is based on phylogenic affiliation, pathways of carbon assimilation, phospholipid fatty acid profile (PLFA) and structure of their internal cell membrane system (Hanson and Hanson, 1996). Type I belongs to the family *Methylococcaceae* with member genera including *Methylosphaera, Methylobacter, Methylomicrobium, Methylosarcina, Methylosoma, Crenothrix, Clonothrix, Methylococcus, Methylocaldum, Methylohalobius, Methylothermus and Methylomonas.* Type II methanotrophs belong to the family *Methylocystaceae* which includes the genera *Methylosinus, Methylocella, Methylocapsa and Methylocystis* (Abell et al., 2009).

Factors such as temperature, oxygen, nitrogen and environmental methane concentrations affect the abundance and growth of different types or groups of methanotrophs living in the

soil environment. Atmospheric methane concentrations in forests soils are considered low and the possibility to take up methane by bacteria (atmospheric methane utilisers) is always constrained by the low levels of carbon and energy source (methane). Studies have shown that Type II methanotrophs phylogenetically closely related to *Methylocystis* and *Methylocapsa* seem to dominate in forest soils and may be responsible for the oxidation of methane at atmospheric levels. However, some other members of the γ- and α- proteobacteria have also frequently been identified in forest soils as separate groups of unidentified methanotrophs (Dunfield et al., 1999; Dunfield & Conrad 2000; Kolb et al., 2003, 2005b).

1.3. Multi-carbon compounds supporting growth of methanotrophs in forest soils

Dunfield et al. (2003) isolated two similar strains of *Methylocella,* named *M. sylvestris,* from acidic forest soils which were discovered to grow at pH 5.5. Dedysh et al. (2005) isolated one strain that could grow on different multi-carbon compounds such as acetate and other simple carbon compounds as the sole carbon source. Thus, the capability of these methanotrophs to oxidise atmospheric methane may possibly be enhanced by the presence of lignin derivatives such as acetate, vanillic acid and guaiacol, which are readily available and abundant in forest soils.

Lignin is a recalcitrant compound and is one of the most abundant organic polymers found in the biosphere. Its decomposition is slow in nature where it is degraded aerobically by fungi into several easily accessible biological compounds such as water-soluble sugars and phenolic compounds. The decomposition process of lignin is characterised by oxidation followed by depolymerisation. The result is the release of individual phenolic substances which can be metabolised by other microbial cells (Wolf and Wagner, 2005). In the decomposition process, compounds such as acetate and vanillic acid are released into the environment.

1.3.1. Acetate

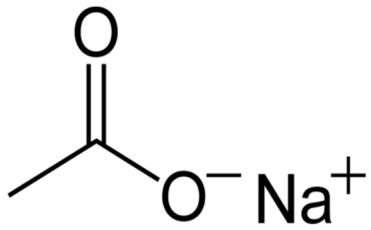

Figure 2: Chemical structure of sodium acetate

Acetate is a two-carbon compound present in forest soils and formed as intermediates at degradation of lignin containing materials. A study by Dedysh et al. (2005) revealed that members of Type II methanotrophic bacteria of the genus *Methylocella* were able to grow solely on methane, methanol or multi-carbon compounds like acetate, pyruvate, succinate, malate and ethanol. The study also showed that this genus of bacteria is able to utilise acetate or methane as its sole carbon and energy source.

Acetate utilisation in the soil environment is deemed vital for the survival of methanotrophs in regions where methane concentrations are limiting or inconsistent. However, acetate cannot solely satisfy the growth requirements of these methanotrophs but permits them to sustain their existence in periods of scarcity of methane and quickly respond when it becomes available again in the soil environment (Belova et al., 2011).

Evidence provided by West and Schmidt (1999) also indicated that atmospheric methane oxidation in tundra soil is stimulated by the addition of acetate.

1.3.2. Vanillic acid (4-Hydroxy-3-methoxybenzoic acid)

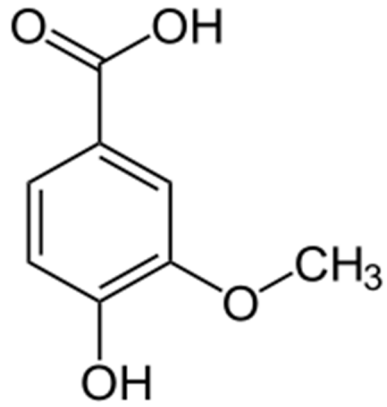

Figure 3: Chemical structure of vanillic acid

Vanillic acid is a naturally occurring phenolic acid found in plant cells and is exuded by living roots. Vanillic acid is the oxidised form of vanillin, a microbial breakdown product of ferulic acid, which in turn is an intermediate product in lignin degradation. It has been identified in forest soils in concentrations of about 0.12 % (Buurman et al., 2007).

1.3.3. Guaiacol (2-methoxyphenol)

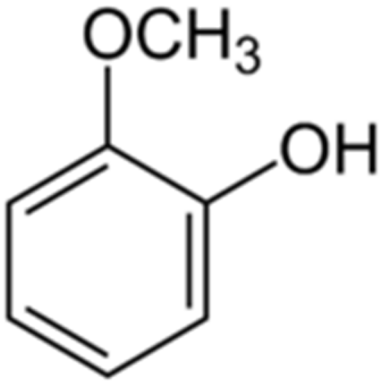

Figure 4: Chemical structure of guaiacol

Guaiacol is a multi-carbon organic compound resulting from the pyrolysis of lignin and serves as a substrate for biosynthesis in a variety of organisms. Organisms such as *Leptoglossus phyllopus* (hemiptarian), *Oxidus gracilis, Pseudopolydesmus erasus* and *Euryurus maculatus* (millipedes) have been shown to biosynthesise both guaiacol and phenol

(Duffey et al., 1977). The guaiacol present in forest soils may serve as an alternative substrate for some organisms.

1.4. Michaelis-Menten kinetics

Michaelis-Menten kinetics is a model that describes the behaviour of an enzyme and describes the rate of reaction in relation to the substrate concentration. The effect of the substrate concentration on an enzyme-catalysed reaction can be described by a hyperbolic relationship between the reaction rate and the substrate concentration. Bender and Conrad (1992) showed that, a similar relationship (Michaelis-Menten kinetics) for consumption of methane in upland soils could best describe the rate of the enzymatic reactions.

In Michaelis-Menten kinetics, the maximum reaction, V_{max}, is defined as the rate when the enzyme is fully saturated with the substrate and its constant K_m (Michaelis-Menten constant), also called enzyme's affinity for its substrate, represents the rate at half the V_{max}. A higher K_m value of an enzymatic reaction represents a low affinity for the substrate and vice versa. An illustration of the Michaelis-Menten kinetics is shown in Figure 5.

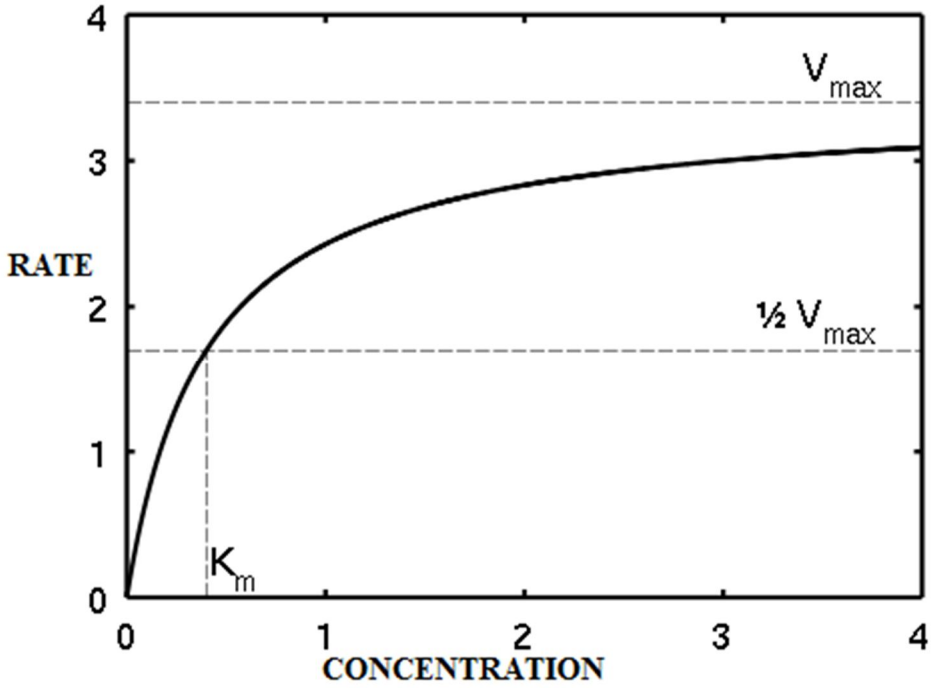

Figure 5: A typical Michaelis-Menten curve of an enzymatic reaction

Studies conducted by Bender and Conrad (1994) showed that all samples obtained from various oxic soils and fresh water sediments exhibited methane oxidation activity below a methane concentration of 2 ppmv, thereby acting as sinks for atmospheric methane. Analysis of methane oxidation in those oxic soils indicated that all soil samples oxidised methane at atmospheric mixing ratios. Another study showed that cambisols had a higher threshold for

methane oxidation and lower K_m values than landfill cover soils (Bender and Conrad, 1992). Boreal soils have been observed to have a high methane oxidation capacity (Whalen et al., 1992).

Incubation studies with constant headspace methane gas concentrations have shown that soil methane consumption varies between different tree species at low methane concentrations, with differences that are greater than those observed in field experiments (Menyailo et al., 2010). However, plant species are known to strongly influence the methane oxidation rates in soils (Borken et al., 2003; Menyailo and Hungate, 2003; Reay et al., 2005; Borken and Beese, 2006).

Information on the influence of multi-carbon compounds on growth of methane oxidising bacteria and oxidation rates is scarce. However, studies by Dedysh et al. (2005) have revealed that, some species of methane oxidising bacteria possess the ability to grow solely on some multi-carbon compounds.

2. AIMS AND OBJECTIVES

The overall aim of this Master's thesis was to provide an in situ understanding of factors governing methane fluxes in the soil and atmosphere. Specific objectives were to:

1. Determine the methane oxidation rate in three typical Swedish forest soils.
2. Establish the effect of tree species on methane oxidation rate in the selected soils.
3. Investigate the effect of other multi-carbon substrates on methane oxidation rate in forest soil.

The work was intended to provide information on the capability of the soil methanotrophs in the kinetics of methane oxidation in three Swedish forest soils and the effect of Scots pine *(Pinus sylvestris),* Norway spruce *(Picea abies)* and birch *(Betula pendula)* on methane consumption rate. It also sought to provide information on the potential effect of other multi-carbon substrates in terms of enhancing the oxidation rate of atmospheric methane or whether methanotrophs are limited to single-carbon bonds.

3. MATERIALS AND METHODS

3.1. Study area

The study area Vipängen is located at Ultuna, south of Uppsala (59°49´N, 17°39´E) and constitutes part of the temperate zone in Sweden. The soil in the study area is classified as a Dystrocryept with the O horizon stretching from the soil surface to a depth of 5 cm, A horizon (5-20 cm) and B horizon (20-40 cm) according to Sundh et al. (2000).

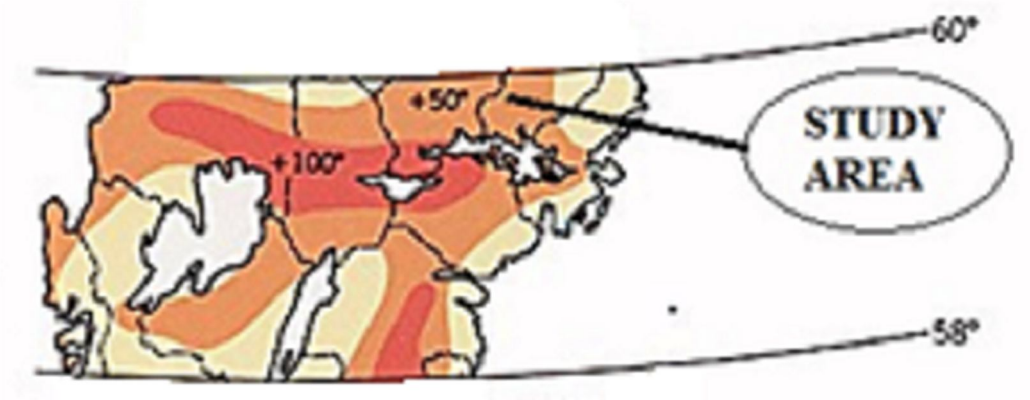

Figure 6: Map showing the study area with coordinates and climate zones (adapted from Swedish National Forest Inventory maps)

3.2. Sampling

On the 22nd of January 2012, three soil samples were collected from three sub-sites at Vipängen. The selection of these sub-sites was based on the dominant tree species being a stand of pine, spruce and birch. After identification of a suitable site, about 1.5 kg soil was excavated with a shovel and bagged in transparent plastic bags.

At sampling, the humus layer which comprised approximately 4 cm depth on the soil surface was removed and samples were obtained from a depth of about 5-10 cm (Figure 7). Sampling was performed within the top soil of the soil profile, which generally represents the zone with the largest rate of methane oxidation in mineral forest soils, as described by Menyailo et al. (2008).

(A) *(B)*

Figure 7: (A) Photo of the study sub-site, after removal of the humus layer during the sampling process.

(B) Photo of the soil samples in transparent plastic bags immediately after sampling.

Upon arrival at the laboratory, samples were sieved with a 4 mm screen and larger organic materials were removed after which the soils were stored cold in plastic bags (+5 °C) for about 3 days before analysis.

Soil pH was determined with a pH meter after dissolving 3 g soil in 10 mL deionised water and allowing it to stand for 6 h. Gravimetric soil moisture and ash content of the soils were determined by drying about 1 g of each sample in an oven at 105 °C for 18 h (dry weight) and by incinerating in an oven at 550 °C for 4 h (ash weight). The resulting data are summarised in Table 1.

3.3. Incubation Experiment

3.3.1. Methane oxidation rate

Five concentrations (4.5, 7.0, 14.1, 45.1 and 300 ppmv) of methane in ambient air were prepared in serial dilutions (118 mL bottles) for incubation.

About 150 g of each soil sample were weighed into 1000 mL air tight Duran flasks sealed with rubber stoppers for incubation. Four replicates of each soil sample and control samples were prepared, in total 20 samples (pine: 5, spruce: 5, birch: 5, and control: 5).These soil samples were incubated with different concentrations of methane taken from the 118 mL bottles.

All soil samples were incubated in a dark environment at room temperatures (about 20-22 °C) throughout the experiment. Incubation carried out was done over a period of 30 h, during which headspace gas samples were withdrawn at different times from the incubation flasks into tight gas vials for gas chromatography (GC) analysis. The consumption of methane over

time was measured for the five different initial concentrations of methane in the gas headspace of the different incubation bottles.

3.3.2. Effect of multi-carbon compounds on methane oxidation

10 g of soil samples was weighed and carefully placed in a 100 mL air tight Duran flask sealed with rubber stoppers for incubation at 5 °C. Three replicates of each substrate and control samples (no addition of substrates) were prepared to obtain 12 flasks in total.

At start, 10 mL of distilled water was added to the guaiacol, sodium acetate and vanillic acid treatments to obtain slurries. The soil to water mixture was chosen to obtain a high efficiency of methane uptake as methane oxidation is negatively affected by water stress in very dry conditions (Whalen et al., 1990; Nesbit and Breitenbeck, 1992; Schnell and King, 1996; Priemé and Christensen, 1999). Castro et al. (1995) showed that an increase of about 60% to 100% water-filled pore space (WFPS) moisture content will reduce methane consumption rate from 130 µg CH_4 $m^{-2}h^{-1}$ to limits below detection level which may be attributed to the fact that methane diffusion into soil is restricted by the availability of water (Ball et al. 1997; Del Grosso et al., 2000) and the creation of anaerobic environments that may reduce aerobic methanotroph communities in soil.

Figure 8: Batch of sample soils in 100 mL air tight Duran flasks ready for incubation.

Stock solutions of each substrate containing the different multi-carbon substrates; sodium acetate, vanillic acid and guaiacol were prepared and kept refrigerated at +8 °C until use. At start of each of the seven repeated measurements 1 mL fresh stock solutions were added.

During each round of incubation the flasks were kept at room temperature on a rotary shaker at 180 rpm. This ensured uniform distribution and good diffusion of methane in the soil slurries. All flasks were aerated at start of each incubation round and then methane was injected to give a headspace concentration of 5-10 ppm (≈ 6.5 ppm), which should be suitable enough to show a significant consumption rate. Gas was withdrawn from incubation flasks at end of each incubation round of 3 days into labelled gas vials for GC-analysis. At the end of each incubation round, flasks were aerated again and replenished with the initial

concentrations of methane to prevent the build-up of anaerobic environments which may eventually affect the growth of the methanotrophs in the soil. The incubation process and GC-analysis were then repeated for all flasks.

3.4. Gas chromatographic analysis

For GC-analyses in the methane oxidation rate experiment, calibration was done by means of five known standard concentrations of methane: 2.01, 9.96, 20, 349 and 1869 ppm. The samples in the gas vials were fed to the GC by a head space sampler and then analysed on a GC with a flame ionisation detector (GC-FID) following the methods of Patel et al. (2011).

The gas sample vials from the multi-carbon experiment were analysed as above but with a known standards of 2.01 and 9.96 ppm methane.

3.5. Methane oxidation rate

Methane oxidation rate was calculated by fitting rates to the Michaelis-Menten equation $\left[v = Vmax * \frac{C}{(Km+C)}\right]$ which best described the relationship between the uptake rate of methane in soils samples. Regression analysis was used to calculate the consumption rates of the various soil treatments on the data from the GC after incubating with different concentrations of methane. The consumptions were expressed on a graph by plotting the concentration (ppm) with time (hour) which gave a curve that best fitted and described the consumption rate. Rearranging Michaelis-Menten kinetics to obtain a linear relationship, the so called Lineweaver-Burk or double reciprocal plot,$\left[\frac{1}{v} = \frac{1}{Vmax} + \frac{Km}{vmax} * \frac{1}{c}\right]$ where V is the reaction rate and C is the initial concentration of the methane, was used to permit more precise fitting to the data and estimations of the V_{max} and the K_m values. In the Lineweaver-Burk plot also called double reciprocal plot, $\frac{1}{v}$ was plotted against $\frac{1}{c}$ where V is the reaction rate and C is the initial concentration of methane.

The effect of the different multi-carbon compounds on the methane oxidation rate was analysed by calculating the methane consumed between start and 72 h of incubation $\left[\frac{\Delta C}{\Delta T}\right]$. This gave a graph that described the relationship between the different addition rates of the multi-carbon compounds on methane consumption rate.

4. RESULTS

4.1. Soil characteristics

Soil pH, dry weight (g), ash weight (g) and organic matter content (%) of the pine, spruce and birch soils are shown in Table 1. Soils obtained from the birch stand had the highest pH of 5.61 and the lowest dry weight of 0.71 g whereas pine had the lowest pH of 4.79 and recorded the highest dry weight of 0.91g. Values obtained from the spruce soil showed a pH of 4.92 and dry weight of 0.84 g. The observed percentage of organic matter content was highest in the birch soil, followed by the spruce and then the pine soil of 22.54 %, 11.90 % and 9.89 % respectively.

Table 1: Some characteristics of the forest soils used in the experiments, originating from pine, spruce and birch

Samples	**pH**	**DW** (g/g wet wt.)	**AW** (g/g dry wt.)	**OMW** (g/g dry wt.)	**OM** (%)
Pine	4.79	0.91	0.82	0.09	9.89
Spruce	4.92	0.84	0.74	0.10	11.90
Birch	5.61	0.71	0.55	0.16	22.54

DW = Dry weight, AW = Ash weight, OMW = Organic matter weight, OM = Organic matter content

4.2. Kinetics of methane oxidation

Methane oxidation was observed in all three soils incubated with different concentrations of methane. The resulting data described a typical Michaelis-Menten curve (not shown). The use of more precise fitting for the data yielded the extrapolation of Michaelis-Menten kinetics to form a Lineweaver-Burk plot, as shown in Figure 9.

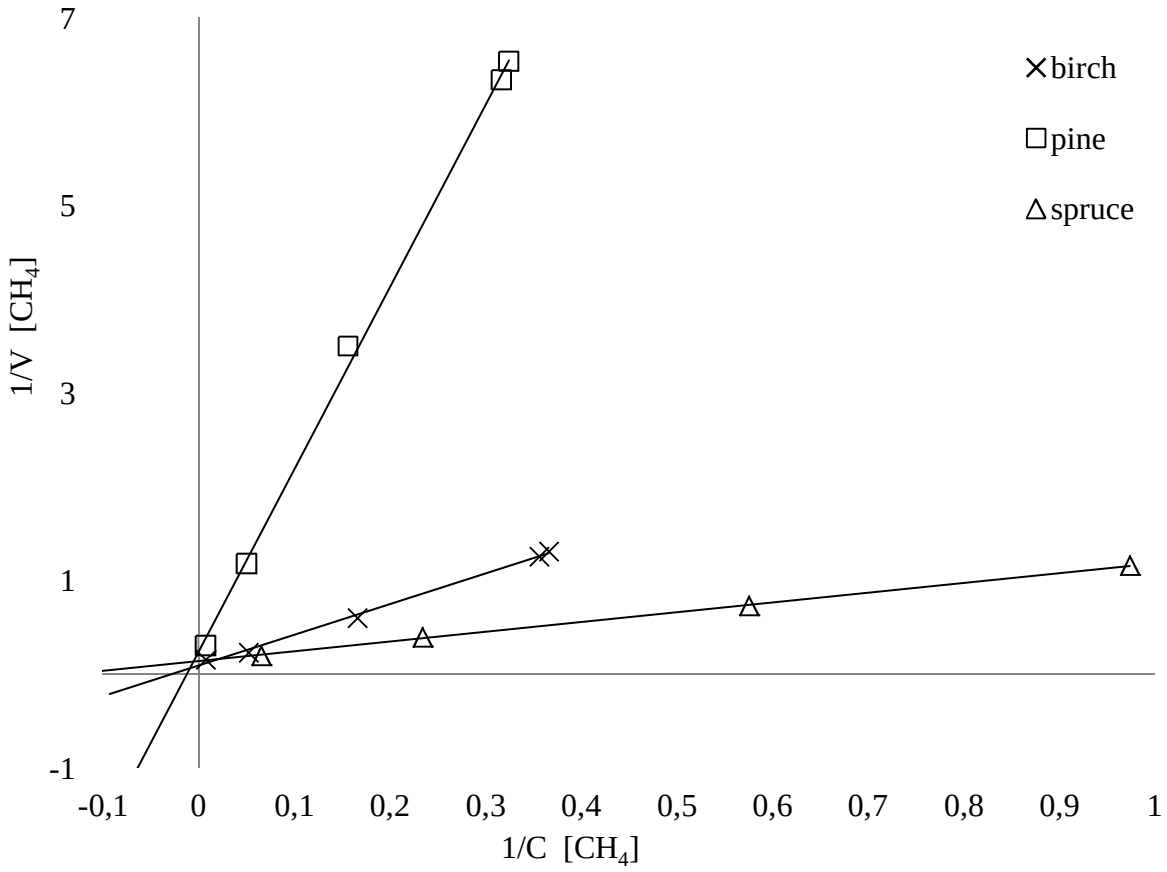

Figure 9: Lineweaver-Burk plots of methane oxidation in forest soils from pine, spruce and birch stands incubated with different concentrations of methane.

According to the V_{max} estimations derived from the point of intercept on the y-axis in the Lineweaver-Burk plots, oxidation of soil samples under the birch stand yielded the highest V_{max} value of 11.0 nmol g^{-1}d.w. hr^{-1}, while the lowest V_{max} value of 4.3 nmol CH_4 g^{-1} d.w. hr^{-1} was obtained in the pine stand soil samples. The maximum oxidation rates (V_{max}) and the apparent K_m values expressed on a dry weight basis for the pine, spruce and birch soils are presented in Table 2.

Table 2: V_{max} and K_m of the plotted Lineweaver-Burk plot of pine, spruce and birch stands

Coefficient	**Pine**	**Spruce**	**Birch**	**Unit**
V_{max}	4.3	7.1	11.0	nmol g^{-1}d.w. hr^{-1}
K_m	185.5	7.4	42.5	nmol g^{-1}d.w.

4.3. Effects of tree species on methane oxidation rates

During the incubation experiments, oxidation at different concentrations of methane per unit volume was subjected to soils of different tree species stand which provided data on their different consumption rates. The consumption rate after 30 h incubation within the three sampled soils types at four initial concentration of methane is shown in Figure 10. The effect of tree species on the methane consumption rate was analysed using two-way ANOVA (from

MINITAB), with tree species and methane consumption rates as independent variables. It was found that tree species had a significant effect ($P < 0.050$) on methane consumption rate.

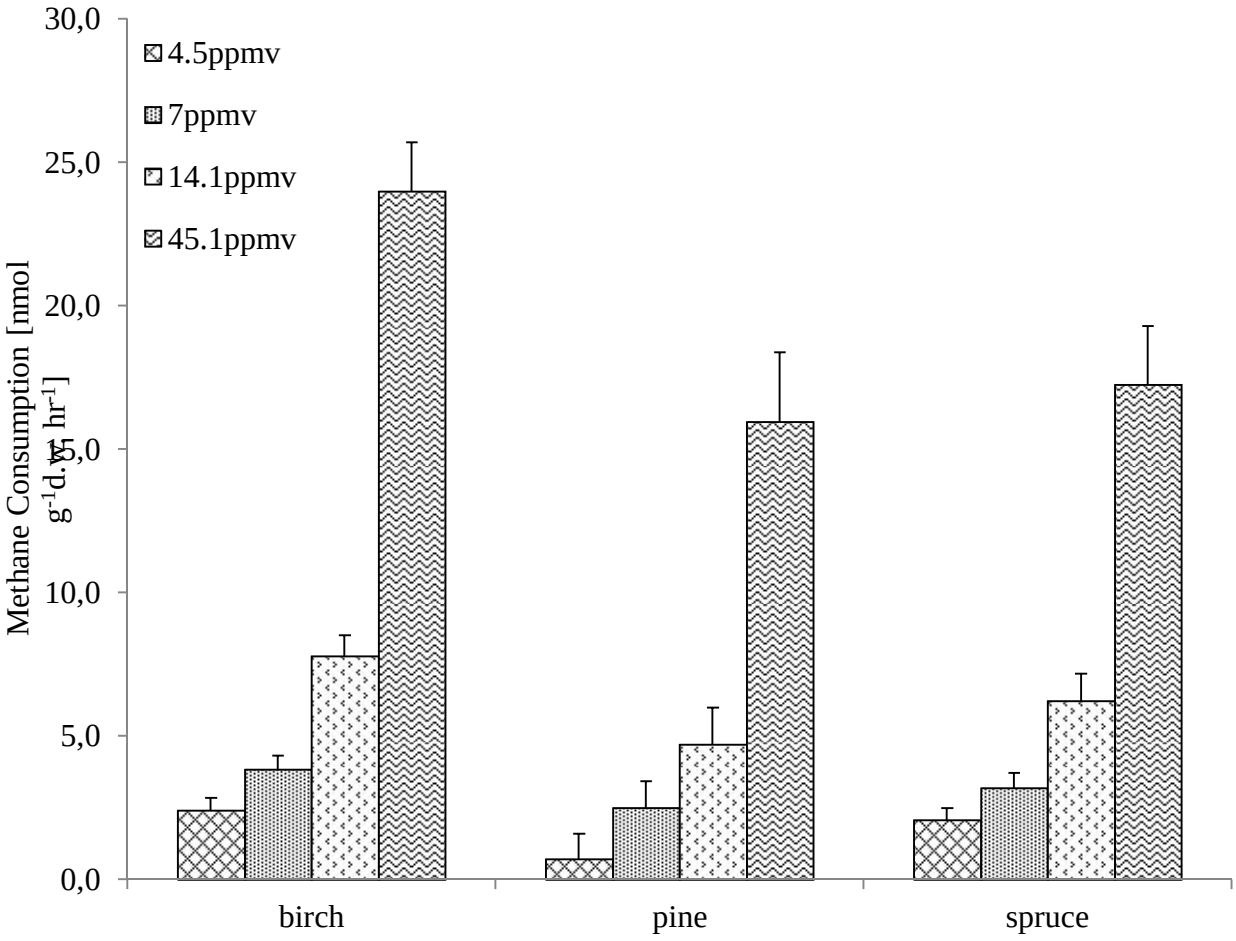

Figure 10: Average methane consumption rate per gram dry weight per hour of three different sampled forest soils of pine, birch and spruce at four different initial methane concentrations (mean and standard error bars)

The highest rates of methane consumption within each methane concentrations were observed in the birch forest soil followed by the spruce and pine soils (birch > spruce >pine). Birch and spruce soil incubated with 4.5 ppmv (2.4 and 2.1 nmol g^{-1}d.w. hr^{-1}) and 7 ppmv (3.8 and 3.2 nmol CH_4 g^{-1}d.w. hr^{-1}) initial concentrations of methane showed similar consumption rates whereas those observed under the pine soils showed the lowest consumption rates of 0.7 and 2.5 nmol g^{-1}d.w. hr^{-1} respectively. Overall methane consumption activities observed at 14.1 and 45.1 ppmv initial concentrations of methane were different for all sampled soils.

4.4. Effects of multi-carbon compounds on methane oxidation rate

The effects of adding the multi-carbon compounds acetate, vanillic acid and guaiacol on the average methane consumption rates in the soils after five weeks of incubation experiment are shown in Figure 11. After each of the seven rounds of the incubation experiment, representing 3 days of incubation with 1 mL addition of each substrate at start (x-axis), the remaining concentration of methane was analysed. The values obtained were then subtracted from the initial methane concentrations to give the methane consumed per gram dry weight of the soil over the 3 days.

After the first incubation round of 3 days, the methane consumption rates in the different substrates did not differ significantly from those with the control samples. However after the second and third experimental rounds, the consumption rate in the acetate-amended soils (39.6 and 39.2 nmol $CH_4\ g^{-1}$ d.w.) showed an increase compared with that in the control samples (36.9 and 25.3 nmol $CH_4\ g^{-1}$ d.w.). In contrast, during the second and third rounds, the samples amended with vanillic acid and guaiacol showed a reduction in methane consumption rate. In the vanillic acid treatments the rate decreased from 28.6 nmol $CH_4\ g^{-1}$ d.w. in the first round to 25.8 nmol $CH_4\ g^{-1}$ d.w. in the second, while in the guaiacol substrates the corresponding decrease was from 30 to 25.5 nmol $CH_4\ g^{-1}$ d.w. From the fourth to the seventh round, all samples showed a declining rate of methane consumption. At the end of the entire experiment only small differences were apparent between the various forest soils and carbon source treatments was observed, with consumption rates ranging between 16.7 and 18.3 nmol $CH_4\ g^{-1}$ d.w. (Figure 11).

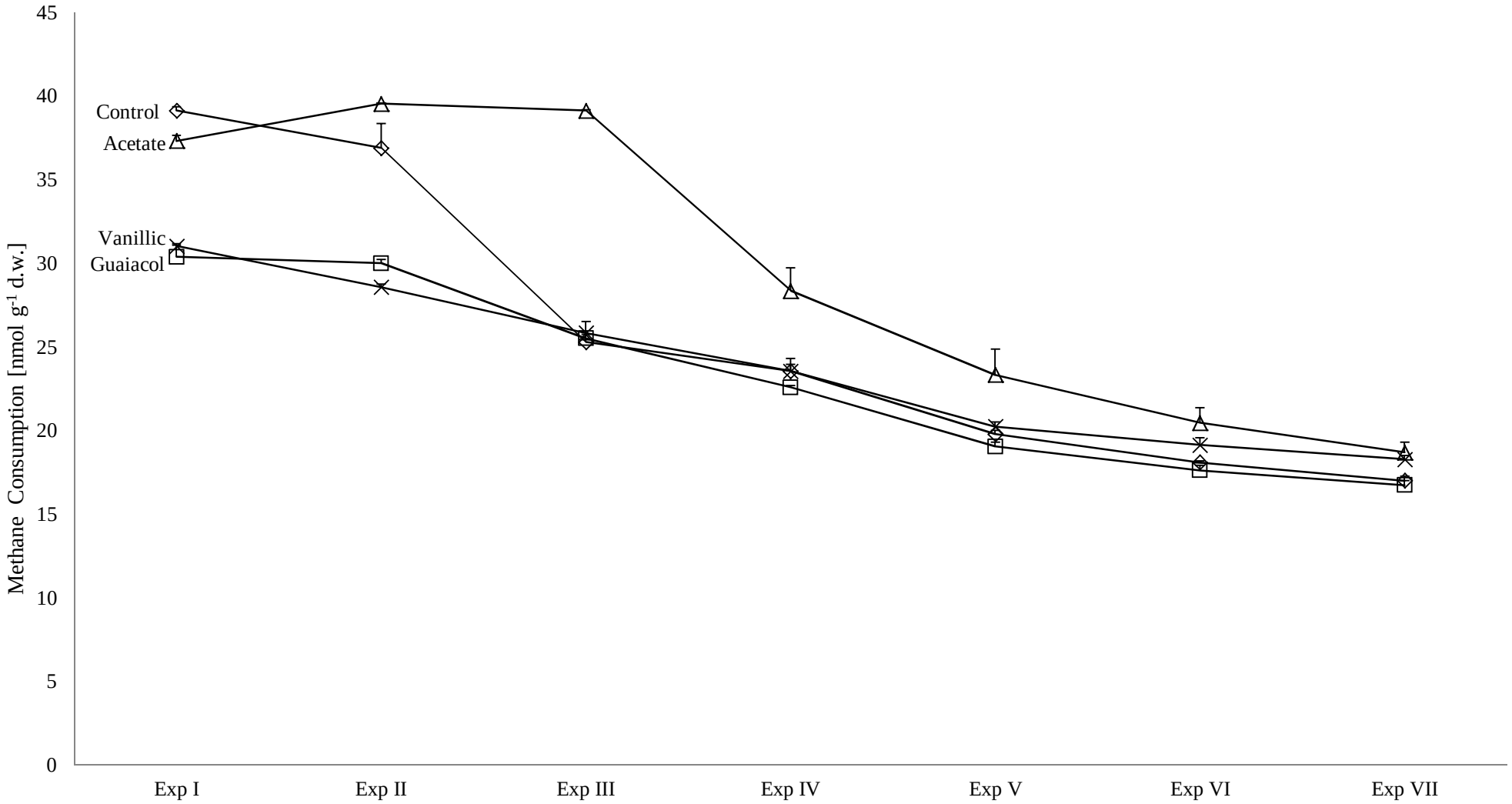

Figure 11: Effect of three multi-carbon compounds on methane consumption rate during 7 experimental rounds of 3 days each. At the end of each incubation experiment, flasks were aerated and replenished with initial concentrations of methane (mean value with standard error bars).

5. DISCUSSION

5.1. Soil pH

The results showed that soil pH had an effect on the initial methane oxidation rate at the start of incubation (pine sample: pH 4.79, methane consumption rate 0.36 nmol CH_4 g^{-1}d.w. hr^{-1} and birch sample: pH 5.61, methane consumption rate 1.9 nmol g^{-1}d.w. hr^{-1}). The pH values observed in this experiment are similar to those reported by the Swedish National Forest Inventory for the period 1993 - 2002 (mean pH value for pine: 4.32, spruce: 4.85, birch: 4.16), except for the birch stand which had higher pH(4.79). However, the effect of pH on methane oxidation rate cannot be fully verified in the present experiment, where the main emphasis was based on the species effect on oxidation rate rather than differences in pH values. Nevertheless, previous studies by Brumme et al. (1999) and Hütsch (2001) support the observation that methane oxidation rate decreases with decreasing pH. Mosier (1998) also reported that decreasing the soil pH from 6 to 4 (by acidifying) in an oxisol reduced the methane consumption rate by 25% but that liming the soil to restore pH could not restore the methane oxidation rate to that observed in the original soils.

5.2. Soil organic matter content

The methane oxidation rate was observed to differ due to the total organic matter content of the soil. Increased soil organic matter content gives higher soil porosity, lower soil bulk density and maintains constant optimal soil moisture content, all of which affect the total methane oxidation rate in soil. An increase in soil porosity increases the rate at which methane diffuses to methanotrophs in the soil. Data obtained from the present study showed decreasing activity in soil in the order birch > spruce > pine, which was correlated to decreasing organic matter content of 22.5, 11.9 and 9.9 % respectively. A previous study by Czepiel et al. (1995) also supports these observations where methane oxidation was influenced by the total organic matter content in soil, within a maximum oxidation rate observed in their case at the highest organic matter content of 14%.

5.3. Kinetics of methane oxidation

Soils exposed to different concentrations of methane for about 30 h yielded information on the methane oxidation kinetics. Linear extrapolation of Michaelis-Menten kinetics to form a Lineweaver-Burk plot was adopted in this experiment to obtain estimates of the K_m and V_{max} values.

The V_{max} values obtained in this experiment ranged between 4.3-11.0 nmol CH_4 g^{-1}d.w. hr^{-1} and the K_m values between 7.4 and 185.5 nmol CH_4 g^{-1}d.w. (Table 2). Activities observed in this study showed a low K_m and V_{max} values which was in the same ranges as those reported by Bender and Conrad (1992) for a forest cambisol and meadow cambisol.

Bender and Conrad (1992) reported variations of high-affinity activities of low V_{max} and K_m values and also low-affinity activities of high V_{max} and K_m values. High-affinity activities was characterised by V_{max} ranging from 2 to 150 nmol CH_4 g^{-1}d.w. hr^{-1} and K_m in a range from 13

to 470 mM whereas low-affinity activities were characterised as V_{max} = 270-3690 nmol CH_4 g^{-1}d.w. hr^{-1} and K_m between 1740 and 27900 mM.

Comparing the K_m and V_{max} values of all three forest soils obtained in the present experiment (Table 2), with those reported by Bender and Conrad (1992), we can classify the methanotrophs under these soil experiments as high-affinity methanotrophs.

5.4. Effect of tree species on methane consumption rate

The second of the three objectives in this thesis was to determine the effect of tree species on methane consumption rate in forest soils. Tree species or forest cover type are reported to have a strong influence on the methane oxidation rates in soils (Borken et al., 2003; Menyailo and Hungate, 2003; Reay et al., 2005; Borken and Beese, 2006).

Borken et al. (2003) observed that methane consumption rates were two to three times higher in beech stand soils than in both pine and spruce forest stand soil in Germany. Menyailo and Hungate (2003) found higher methane oxidation rates under Siberian hard wood species (aspen and birch) than those observed in coniferous species soils and grassland adjacent to forest soil. Similarly, the studies by Reay et al. (2005) and Borken and Beese (2006) showed that tree species influences the methane uptake rate in soil, with higher uptake rate under beech stands than under spruce forest stands in Germany by Borken and Beese (2006). Oak, Norway spruce and Scots pine vegetation were found to be key determinants of methane uptake rates in a study measuring high and low oxidation potentials for five replicated land-use treatments over the entire year in Bowland Forest in Lancashire, UK (Reay et al., 2005). These overall findings were confirmed by the results from the present incubation experiment, in which the different forest soil samples showed significant differences ($P < 0.050$) in methane consumption rate in a similar environment with different concentrations of methane.

Methane consumption rates observed in Swedish forest soils under birch, pine and spruce stands and in Siberian forests with similar species by Menyailo et al. (2010) were comparable to the consumption rates observed here, with birch soils displaying the highest consumption rates in both studies. The Swedish forest soils yielded a consumption rate order: birch > spruce > pine under 45.1 ppmv; while the order observed in Siberian forests was: birch > pine > spruce under 30 ppm of methane. The differences in consumption rates between pine and spruce species may be due to the geographical area and the different biological activities within the different soil environments, yielding the growth of different methanotroph communities (Degelmann et al., 2010).

The effect of tree species on methane consumption rate may also be associated with the fact that plant species diversity increases the composition of the soil microbial community (Zak et al., 2003) or its microbial diversity (Groffman and Bohlen, 1999; Nannipieri et al., 2003) thereby supporting methanotrophs that may have a high affinity for their substrates.

5.5. Effect of multi-carbon compounds on methane oxidation rates

Acetate-containing samples increased the methane consumption rate within the Swedish forest soils tested here compared with the control soils, an effect which has also been observed in alpine tundra soils (West and Schmidt, 1999). However in the present study, subjecting soil samples to additions of vanillic acid and guaiacol did not give any positive response on the overall methane consumption rates. Therefore it did not stimulate any methane consumption rate in Swedish forest soils tested throughout the entire incubation period.

Towards the end of the incubation experiment (rounds six and seven), the consumption rate within all sampled soils including acetate-containing soils showed methane oxidation rates below 20 nmol $CH_4\ g^{-1}$ d.w. This effect was probably caused by depletion of some soil micro- and macronutrients due to the fact that soils subjected to incubation for some periods of time are prone to nutrient depletion, thereby leading to inhibition of growth of the soil microorganisms, including the methanotrophs. The possibility of methanotrophs interacting with fungi and other microorganisms and competing for nutrients can also explain this effect.

5.6. Effect of some inhibitory substances on methane oxidation rate

The presence of monoterpenes in forest soils is said to have an inhibitory effect on the methane oxidation rate that is directly dependent on the amount of substance (monoterpenes) available in the soil environment.

Current studies show that, methanotrophs and some denitrifying bacteria are known to be more sensitive to monoterpenes than other bacteria (Amaral and Knowles, 1998). Hence, their sensitivity (hindering their growth and activities) is of great importance as it might be an explanation for the low uptakes of methane in some forest soils and can explain what was observed within this study.

6. CONCLUSIONS

This laboratory study showed that, methane oxidation in soil samples from Swedish birch, spruce and pine forest is attributable to high-affinity methanotrophs present in the soil environment. The presence and oxidation activities of these methanotrophs are limited by low soil pH and low total organic matter. Tree stand species may also affect the ability of the methanotrophs to consume methane.

The multi-carbon substrate acetate increased methane oxidation rate from the three forest soils studied, thus increasing its effect on the atmospheric methane sink. Addition of vanillic acid and guaiacol did not show such an effect. Further investigations are needed to fully determine the effects of other substrates on methane consumption rates.

Soil organic matter is probably the most important variable in methane oxidation and the organic matter content in agricultural soils is only, half that in forest soils. The depth of the litter layer (and incidence of degrading fungi) also differs between forest soils in temperate and tropical regions.

7. RECOMMENDATION

Stable isotope probing (SIP) for RNA and DNA molecular analysis would be more suitable for characterising the structure and in situ function of the active methanotroph populations available on soil samples obtained. This method could also reveal the substrate degradation pathways and the interactions between microorganisms. The use of large numbers of replicate samples would also give a broader perspective on the effect of soil pH on oxidation processes in Swedish forest soils. Furthermore, the process of atmospheric methane consumption is attributed to methanotrophic communities of different species thriving in different environments, but their existence in forests soil has not yet been fully studied. In situ analysis on the physiology of some methanotrophic species (culturable and unculturable) would pave way for a meaningful understanding of the soil as an atmospheric methane sink.

Investigations using other multi-carbon compounds found in forest soil floors might also provide insights into the effect and importance of some other multi-carbon compounds on the growth of some methanotrophic bacteria and thus on overall atmospheric methane fluxes.

7.1. Final remarks for forest management

The increasing demand for wood fuel harvesting to support district heating systems in Sweden is resulting in rapid removal of forest residues, which account for about 2.5 % of the total energy (16 %) generated from biofuels in Sweden (Anon, 2002). Tree branches, tops of trunks and tree stumps, which are the main sources of lignin components in the forest environment, are under pressure as large amounts are required to satisfy the increasing utilisation of bioenergy.

Although the use of forest residues for bioenergy poses a lot of environmental advantages, their removal from the forest environment causes a great reduction in the total number of lignin degradation substrate. This in turn decreases the amount of lignin derivatives, which were shown in this thesis to enhance the growth of methanotrophs and atmospheric methane uptake rates in forest soils.

In addition, during forest regeneration after clear-cutting, some consideration must be given to the type of tree species planted. The consumption of some GHG's (methane) by forest soils, which serves as the main removal process on the Earth's surface is affected by tree stand species. It's consideration during tree planting, will enhance the methane consumption rate (birch > spruce >pine), which on a global scale could account for a percentage reduction in global warming.

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APPENDIX

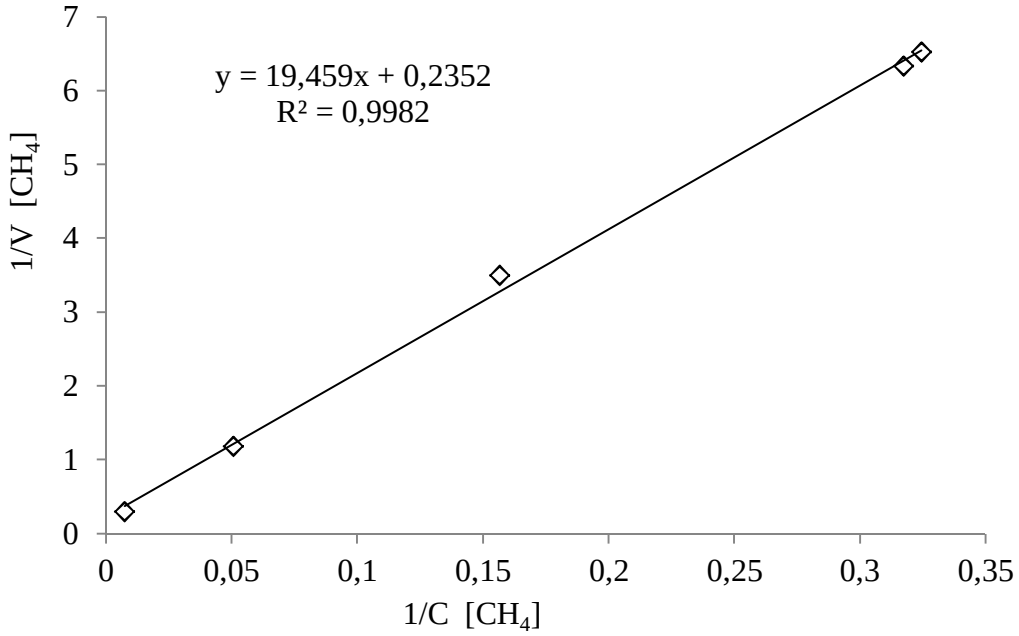

Figure 12: Lineweaver-Burk plots (1/v against 1/c) of methane oxidation rates in a pine forest soil incubated with different concentrations of methane.

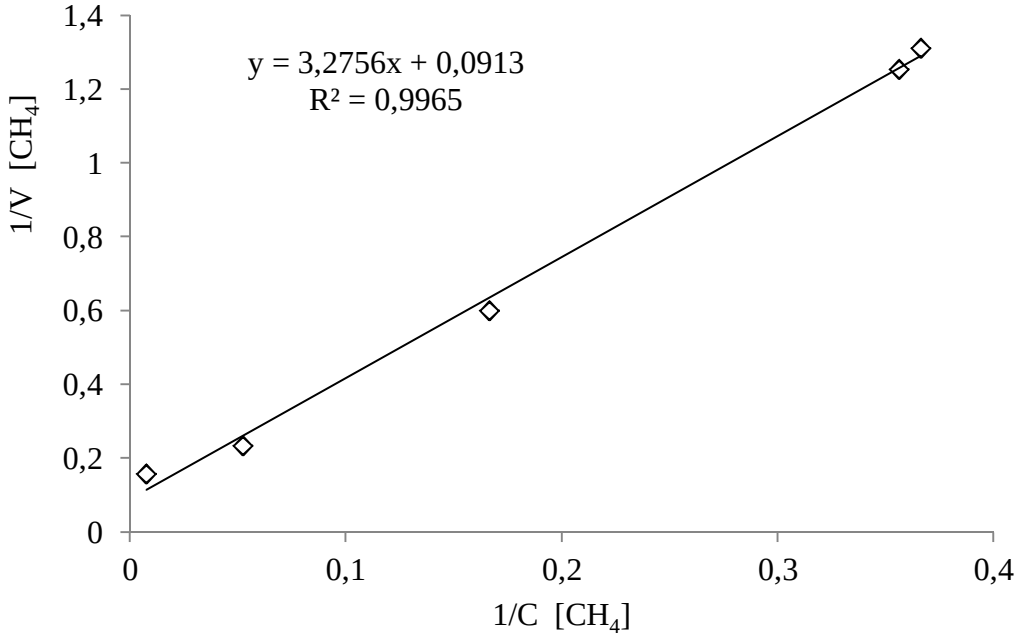

Figure 13: Lineweaver-Burk plots (1/v against 1/c) of methane oxidation rate in a birch forest soil incubated with different concentrations of methane.

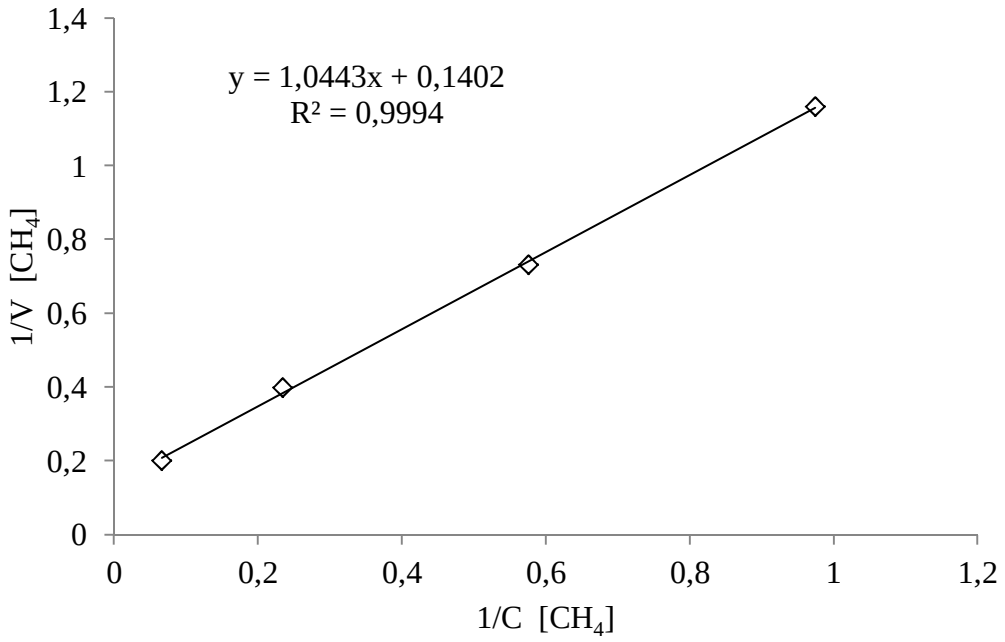

Figure 14: Lineweaver-Burk plots (1/v against 1/c) of methane oxidation rate in a spruce forest soil incubated with different concentrations of methane.