Soil methanotrophs—a novel methane mitigation technology?

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1. Introduction

Methane (CH_4) is a potent greenhouse gas (GHG) produced globally by both biotic and abiotic processes. There is still some uncertainty in the global CH_4 budget, highlighted recently by the discovery of a possible new biotic source in growing plants (Keppler et al., 2006).

The most important known sources of CH_4 are natural wetlands (21%); fossil fuel related to natural gas, coal mines, and the coal industry (16%); enteric fermentation (16%); rice paddies (11%); biomass burning (7%); landfills (7%); and animal waste (5%). The major sinks for CH_4 are biological oxidation at or near the sites of production (~700 Tg y^{-1}), and photochemical oxidation in the atmosphere (~450 Tg y^{-1}). However, oxidation of atmospheric CH_4 by aerobic soils also provides a significant additional sink (20–60 Tg y^{-1}). The net flux between soils and the atmosphere results from the balance between two microbiological processes: methanogenesis (production of CH_4) and methanotrophy (consumption of CH_4). Because the soil sink for atmospheric CH_4 is microbially mediated it is sensitive to environmental factors (e.g., moisture, temperature), and disturbance by management (Reeburg et al., 1993).

Methane oxidation by forest soils, particularly oxidation kinetics, location in soil, and inhibition, has been studied extensively because these soils represent a major part of the soil sink in the global CH_4 budget. The highest CH_4 oxidation activity in forest soils is usually measured in subsurface soil layers (Adamsen and King 1993; Koschorreck and Conrad, 1993; Roslev et al., 1997; Whalen et al., 1992). This localisation of methanotrophs in deeper soil layers has been attributed to inhibition of methanotrophs by ammonium or terpenes present in the organic surface layer of forest soils (Amaral and Knowles, 1997; 1998).

Recently, Saggar et al. (2008) reviewed soil CH_4 oxidation rates measured in New Zealand pastures under cattle- and sheep-grazed pasture, pine and beech forests, regenerating shrubland, and cropland. These datasets suggested all soils oxidise CH_4 but soil oxidation rates vary markedly with land use. Methane oxidation rate was highest for a New Zealand beech forest soil (10.5 kg CH_4 ha^{-1} yr^{-1}). The pine soils had intermediate oxidation rates (4.2–6.4 kg CH_4 ha^{-1} yr^{-1}), while the oxidation rates for all pasture and cropped soils were mostly <1 kg CH_4 ha^{-1} yr^{-1} (Table 1).

Ungrazed and unimproved pasture soils had similar CH_4 oxidation rates to those in improved and intensively managed dairy- and sheep-grazed pasture soils, suggesting increased intensification of agriculture from sheep to dairying has little impact on soil CH_4 sink capacity. On the other hand, the capacity of some soils to oxidise CH_4 may have declined from the effects of high N inputs from fertiliser, dung, and urine. This could imply that, since 1990, the CH_4 sink strength of New Zealand soils has declined, effectively increasing net CH_4 emissions. Deforestation in the North Island to make more land available for dairying is another reason to believe CH_4 oxidation in soil may be decreasing, because this land-use change is causing large increases in N inputs to soil, and these are known to affect soil CH_4 oxidation.

Table 1. Soil methane oxidation estimates for different land cover and land use (from Saggar et al. 2008)

Land Use	Annual consumption ($kg\ CH_4\ ha^{-1}\ yr^{-1}$)	Reference
Native Beech	10.5	Price et al. (2004a,b)
Pine	4.2–6.4	Tate et al. (2006)
Shrub land	2.3	Tate et al., (2007)
Dairy pasture	0.5– 0.6	Saggar et al. (unpublished)
Sheep pasture	0.6–1.0	Saggar et al.(2007)
Ungrazed pasture	0.85	Saggar et al. (2007)
Cropping	1.5	Van der Werden (1999)

However, there is considerable uncertainty about the effects of N on CH_4 emissions, with recent in vitro research (Kruger and Frenzel 2003) suggesting a positive effect of additional N on methanotrophic bacteria for potential CH_4 oxidation rates in soil and root samples. These findings contrast with those of earlier work (Steudler et al. 1989; Mosier et al. 1991) showing negative effects of ammonium-N on CH_4 oxidation. We have found little evidence of an N effect on CH_4 oxidation in New Zealand soils (Tate et al., 2007), indicating that soil aeration status and the nature of the methanotroph population in soils together explain most of the changes observed in CH_4 oxidation with land-use change in New Zealand.

Based on the limited data available, Saggar et al. (2008) estimated a possible increase in national soil CH_4 oxidation due to afforestation since 1990 of 0.8–7 Gg CH_4 that, if proven, could offset 1–8% of increased agricultural emissions during this period. Although small, this offset would nonetheless be useful for national reporting purposes. The wide range of the potential offset is mainly because data are limited.

This report provides new data on soil CH_4 oxidation in reverting shrubland. We also describe new research, using our recently acquired knowledge of the mechanisms regulating soil CH_4 oxidation (Tate et al 2007; Singh et al. 2007), to develop a practical technology for capturing emissions from housed animals, effluent ponds, and landfills. This latter research has been made possible through collaboration with the Macaulay Land Use Research Institute (Aberdeen, UK) and the University of Victoria, B.C., Canada.

2. Methane oxidation in pasture and shrubland soils

2.1 Introduction

Land-use change is accelerating globally in response to a rapidly growing human population. This is increasing pressure on indigenous ecosystems as more land is required for food, housing, and fuels (biofuels) (Reay and Grace, 2007). Clearance of forest and shrubland in the tropics is particularly prominent in the Asia – Pacific region (Zhao et al., 2006). However, in countries that are signatories of the Kyoto Protocol, regeneration of indigenous shrubland and forest coupled with restoration or planting of both native and exotic forest is increasingly seen as a means to reduce GHG emissions and sequester carbon (C). In New Zealand (NZ), 9.9% of the total land area is currently shrubland (Tate et al., 2005). Nationally, around 1.4 million hectares of marginal pastoral land has been identified as highly suitable for shrubland regeneration, based on a high risk of soil erosion combined with nearby shrubland seed sources. This includes large areas of the southern and east coast regions of the North Island and Banks Peninsula and coastal and inland Otago in the South Island (Trotter et al., 2005).

Regeneration of indigenous shrubland can have multiple benefits including C sequestration, soil stabilisation and development on erosion-prone land, and enhancing biodiversity. In addition, recent studies show that enhanced oxidation of atmospheric CH_4 also occurs under regenerating shrubland (Tate et al., 2007). Currently, New Zealand's CH_4 emissions are 6.6% greater than 1990 baseline levels (Ministry for the Environment, 2007), mainly because of agricultural intensification, especially dairying. Therefore, options to mitigate anthropogenic CH_4 emissions are required urgently. Soil CH_4 oxidation is a natural process that provides a limited but useful opportunity to offset increasing emissions. However, field data are scarce for soil CH_4 fluxes from a range of ecosystems, including unimproved pasture reverting to shrubland in New Zealand (Saggar et al., 2007). By providing new data for this land-use change, this study will help refine New Zealand's net GHG emissions budget.

2.2 Methods

The study area was located at Little River on Banks Peninsula (approximately 180 m elevation; Lat. 43.74^{o}S, Long 172.85^{o}E), 70 km SE of Christchurch, NZ. The soil was a Pawson silt loam (colluvium variant) (Trangmar, 1986) or an Aquic Haplustept (USDA soil classification) (T. Webb, Landcare Research, pers. comm. 2008). The experiment utilised three sites on the same property containing regenerating kānuka (*Kunzea ericoides* (A Rich) J. Thompson) shrubland at differing stages of development. Six replicate plots were each located on unimproved pasture (UP), consisting primarily of brown top (*Agrostis capillaris L.*) and creeping bent *(Agrostis stolonifera L.*) and considered as the baseline [plot size ≈ 5 m x 12.5 m]; young kānuka (YK) 8–12 years old and 1–3 m in height [plot size ≈ 12.5 m x 15 m]; and old kānuka (OK) approximately 80 years old, and 4–5 m in height [plot size ≈ 15 m x 15 m]. The regeneration of the OK is thought to have begun after a large flood in 1923 following logging, (Ogilvie, 2007). Animals grazed the UP and YK areas prior to the study, but were excluded once it commenced. The OK site had been undisturbed by stock for at least 6–7 years prior to the study.

The chambers used for measuring CH_4 fluxes at the OK and YK sites were transparent

polyethylene domes (0.17 m diameter, 0.13 m height) and the UP chambers were cylindrical and constructed of PVC (0.24 m diameter, height 0.03 m for increased sensitivity). A gas-tight seal was achieved using permanently positioned chamber bases fitted with a foam margin. The chambers were anchored with metal bull clips. Gas samples were collected and analysed using GC-FID (Price et al., 2004a). Environmental and meteorological variables were measured and recorded using dataloggers. All soil CH_4 flux data are presented as means ± 1 SE.

2.3 Results

Surface CH_4 oxidation rates in the UP, YK, and OK sites averaged -14.2 ± 3.1, -7.5 ± 6.4, and -54.0 ±6.6 μg CH_4 m^{-2} h^{-1}, or -1.2, -0.65 and -4.7 kg CH_4 ha^{-1} y^{-1}, respectively. The negative sign indicates all sites were a net sink for atmospheric CH_4 on an annual basis (Fig. 1); however, sporadic CH_4 emissions also occurred at each site. In early summer at the YK site, CH_4 fluxes reached a maximum of -4.3 kg CH_4 ha^{-1} y^{-1}, suggesting a potential for rapid recovery of CH_4 oxidation rates as soils dried following a period of CH4 emission. Methane flux for the OK site was about half the rate measured in an undisturbed indigenous *Nothofagus* forest soil (10.5 kg CH_4 ha^{-1} y^{-1}) (Price et al., 2004a). The principal environmental variable responsible for regulating soil CH_4 oxidation rates in the UP and OK sites was soil water content at 50 mm depth (Fig. 2). At the YK site, it was not possible to demonstrate any significant relationships between CH_4 oxidation rates and soil water content (and soil temperature) because of high variability in the CH_4 fluxes.

2.4 Discussion

Inter-site comparisons of soil CH_4 oxidation

The average CH_4 oxidation rates for the UP site compare favourably with other datasets in Australasia (Judd et al., 1999, Li and Kelliher 2007, Dalal et al., 2007) and elsewhere (van den Pol-van Dasselaar et al., 1999). Similarly, at the YK site, CH_4 oxidation rates were comparable to those in similar environments including reforested areas (Livesley et al., 2007) and successional forests (Suwanwaree and Robertson 2005), where rates varied from -9.1 to -42.1 ± 4.3 μg CH_4 m^{-2} h^{-1}. Oxidation rates for the OK site can be compared to those for chaparral, a shrubland/heathland plant community with similar vegetation structure (-25.7 μg CH_4 m^{-2} h^{-1} or 2.25 kg CH_4 ha^{-1} y^{-1}, Dutaur and Verchot, 2007). These rates are lower than those observed for the OK site; this is surprising given the OK site occupies a moister, temperate environment.

At the UP and YK sites CH_4 oxidation rates were similar, but much lower (by 4–7 fold, respectively) than the CH_4 oxidation rates for the OK site. Variation in soil type may be partly responsible for regulating these CH_4 fluxes. The variability in the YK data could have resulted from changes in compaction, and, hence, bulk density because the area was previously grazed and sheep trails were present. These tracks are similar to livestock camp areas (Dalal et al., 2007), and are generally used for access; consequently, variability in CH_4 fluxes (with both CH_4 emission and oxidation occurring) may have been due to changes in the soil aeration caused by stock treading.

Impacts of land-use change on CH_4 oxidation

The CH_4 oxidation rates measured at the three sites were all lower than those previously measured in a *Nothofagus* forest soil (10.5 kg CH_4 ha^{-1} y^{-1}) (Price et al., 2004a) but consistent with other sites undergoing regeneration or afforestation. Prieme et al. (1997) found CH_4

oxidation took 100–200 years to recover under a woodland environment following the cessation of agricultural disturbance. During early regeneration, recovery of soil CH_4 oxidation rates tends to be slow, but rates were higher in soil beneath the older shrubs. Roberston et al. (2000) compared soil from a 10-year-old successional ecosystem with an agricultural soil, and found CH_4 oxidation rates were about 10% greater than those of the agricultural soil. In a later study at the same site, oxidation rates recovered to 75 % in wooded plots 40–60 years after agriculture ceased (Suwanwaree and Robertson, 2005). Likewise, Tate et al. (2007) compared soil from pasture with soil from a nearby 50-year-old kānuka shrubland and under laboratory conditions measured an increase in CH_4 oxidation rates of about 50% in the shrubland soil. However, once shrubs and trees become established the pattern of CH_4 fluxes is not always towards CH_4 oxidation. For example, Maljanen et al. (2001) showed that newly afforested (1 and 6 years prior) boreal agricultural peatland soils were a source of CH_4 and became a sink only after 23 years of afforestation.

Conclusion and implications

All sites acted as a net sink for CH_4, with CH_4 oxidation being the highest in the OK site. CH_4 oxidation in young regenerating shrubland soils was more variable, but these soils showed significant CH_4 oxidation rates during warmer and drier times of the year. With the increasing likelihood of more droughts in eastern areas of New Zealand, soil CH_4 sink capacity is likely to increase. Clearly, restoration of soil CH_4 oxidation rates under young shrubland takes time, and more data are required to characterise CH_4 oxidation under shrubland at different stages of development.

Using data from this and previous studies, we can roughly estimate the proportion of national CH_4 emissions that would be offset if the 1.4 M ha of marginal pastoral land identified by Trotter et al. (2005) as suitable for indigenous reforestation were vegetated with established shrubland. The net increase in CH_4 oxidation rate with land-use change from pasture to shrubland is 1.3–4.2 kg CH_4 ha^{-1} y^{-1} (pasture: 0.5–1.0 kg CH_4 ha^{-1} y^{-1}, shrubland: 2.3–4.7 kg CH_4 ha^{-1} y^{-1}). Scaling over 1.4 M ha, and converting to CO_2-equivalent (CO_2-e) units, this equates to 0.038–0.124 Mt CO_2-e y^{-1}. In 2005, national CH_4 emissions were 1.8 Mt CO_2-e y^{-1} greater than in 1990. Thus, reforestation of marginal pastoral land could potentially offset 2.1–6.9% of CH_4 emissions above 1990 levels. This is in addition to carbon storage benefits from reforestation.

As soil CH_4 oxidation clearly has potential for mitigating anthropogenic CH_4 emissions, more baseline research is required to investigate the process in the field in more soil types and ecosystems, particularly in areas likely to be drier in future. A more robust scaling calculation is required, combining process-based models with spatial datasets to take account of the regions and times of the year with the greatest potential for CH_4 oxidation and to quantify uncertainties in the uptake calculation. Once an improved baseline for the size of the soil CH_4 sink in NZ is established, strategies to enhance CH_4 oxidation involving land-use change can be optimized. Similarly, by selecting soils containing active methanotroph populations, it may be possible to develop methane biofilters for particular applications on farms and landfill sites, as Section 3 demonstrates.

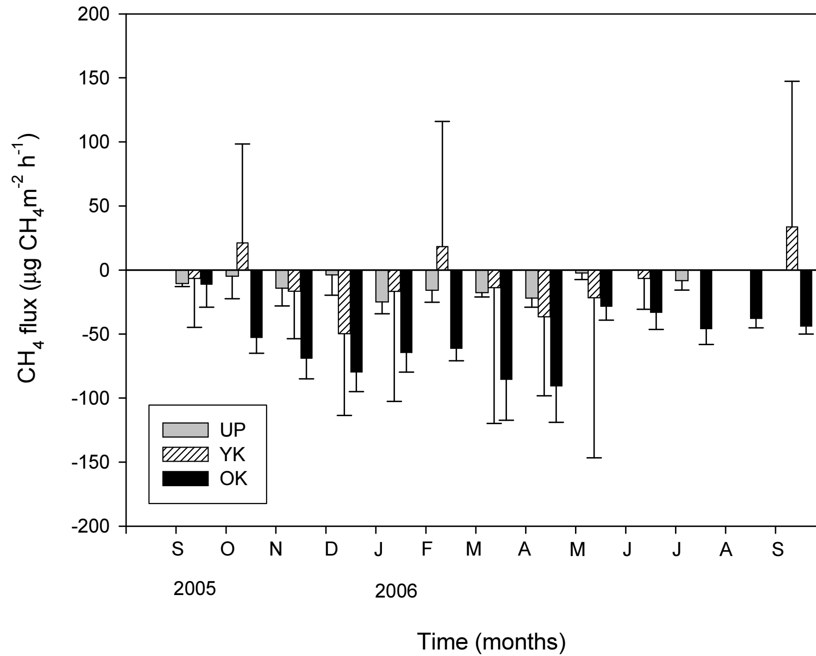

Fig. 1 Soil CH_4 fluxes in a regenerating kanuka sequence. Notation: UP (unimproved pasture), YK (young kanuka, 8–12 years old), and OK (old kanuka, about 80 years old). Negative values indicate net methane uptake. Periods in the year where no data are shown indicate fluxes below the minimum detectable flux for the equipment. Error bars are 1 SEM.

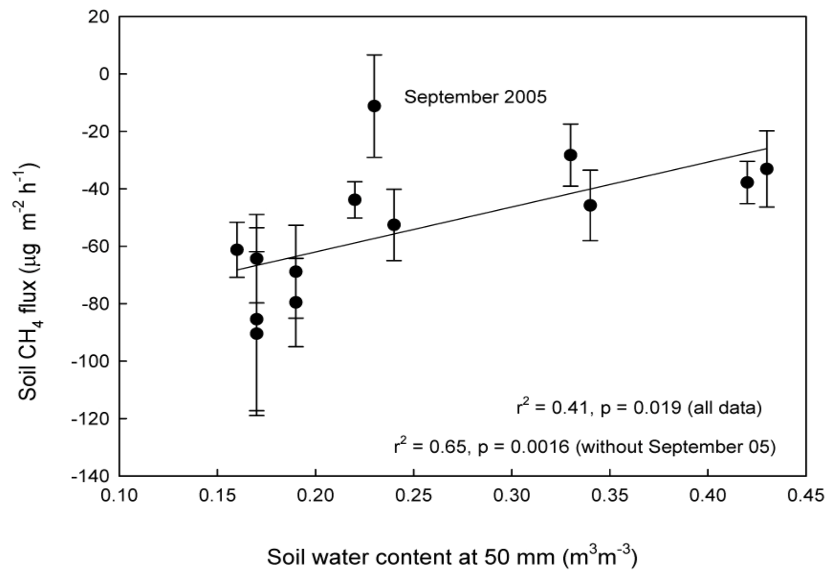

Fig. 2 Relationship between soil CH_4 fluxes and water content in the OK. Note that in September 2005 some CH_4 emissions occurred. Error bars are 1 SEM.

3. Methane oxidation by soil methanotrophs—a potential biofilter?

3.1 Introduction

Methane (CH_4), primarily from ruminant animals, accounts for 35% of New Zealand's total GHG emissions (Ministry for the Environment, 2007). Despite the large contribution from enteric fermentation, no mitigation options are currently available apart from reducing livestock numbers, which would have a large negative impact on farmer's livelihoods and the economy as a whole. Consequently, a major research effort currently aims to reduce the production of CH_4 in the rumen by manipulating its methane-producing bacteria. Another option could be to "treat" the CH_4 (i.e. oxidise it to carbon dioxide and water vapour) after it has been emitted by the animal, but clearly this is not feasible when the animals are grazing in paddocks.

However, livestock (particularly dairy cattle) are increasingly being contained in small areas for long periods. There is increasing recognition of the production benefits of removing cattle from paddocks when soil and pasture damage is likely. It is now common for animals to be removed from pasture to specially constructed pads for up to 20 hours per day during winter and early spring. Further, many farmers are recognising animal welfare and production benefits of providing shelter in the form of partial-housing (e.g., "herd homes") or full-housing when animals are removed from pasture. Such shelters are also used at other times of the year to feed out supplements with minimal wastage. When ruminant animals are housed together, the concentration of CH_4 in the surrounding air rises and "treating" the emitted CH_4 becomes more feasible.

Methane is also emitted from animal waste management systems, mainly dairy effluent treatment ponds. This source (0.8 Mt CO_2-e y^{-1}) comprises only around 3% of total national CH_4 emissions but is growing rapidly (28% since 1990, Ministry for the Environment, 2007) in line with the recent expansion of dairy farming. Using existing technologies it is possible to cap effluent ponds, capture the emitted CH_4, and use it to generate electricity. However, on smaller farms this is unlikely to be economically feasible given the large capital expenditure required. In these situations, a low-cost means of reducing CH_4 emissions from effluent treatment ponds would be valuable.

Biofilters have been used for several decades to treat exhaust air with low concentrations of contaminants, such as odorous air from wastewater, composting plants, and animal housing (Streese et al., 2005). A biofilter comprises a porous medium on which odour-removing bacteria grow. Air is passed through the filter and contaminants are converted to carbon dioxide, water vapour, and organic biomass. Methane biofilters have also been developed that utilise soil methanotrophic bacteria to oxidise higher concentrations of CH_4 emitted from landfills (Nikiema et al., 2007). We believe it may be possible to adapt this technology and use soil methanotrophs to filter CH_4 emitted from housed ruminants and effluent storage areas.

In this project we describe an experiment designed to quantify the methane oxidation potential of soil methanotrophs sampled from two different land uses: planted forest and pasture. Soil CH_4 oxidation under forest is significantly greater than under pasture (Tate et

al., 2007) reflecting differences in soil moisture, bulk density, and porosity. However, methanotroph populations differ under forest and pasture vegetation (Singh et al., 2007), and this also contributes to the observed difference in CH_4 oxidation rates. Given that animals may be absent from housings for long periods, in this preliminary study we also examine the response of methanotrophs to large variations in CH_4 concentration from ambient (about 1.8 ppm) to 600 ppm.

We hypothesise that CH_4 oxidation in the forest soil will be greater than in the pasture soil and that the presence of different methanotroph populations can explain much of these different responses. In both soils we expect the microbes to be substrate-limited, so that CH_4 oxidation will increase rapidly in response to an increase in CH_4 concentration. Prolonged exposure to elevated CH_4 may stimulate growth of these methanotroph populations and further increase CH_4 oxidation rate.

3.2 Methods

Soil sampling

Samples of soil were collected from Puruki, in the Purukohukohu Experimental Catchment. The site is in the central North Island at 38º36'S, 176º13'E, where soils are Oruanui sandy loams (Andisols) formed in pumice about 1800 years old. Between 1976 and 1985 average annual rainfall at the site was around 1500 mm (range 1150–2010 mm) and average annual air temperature varied between 9 and 11 ºC (Beets and Pollock, 1987). Two sites in close proximity on the same soil type were resampled in the same areas as those used in our original study (Tate et al., 2007). The first site was in pasture previously grazed by sheep and cattle, where vegetation consisted of ryegrass, cocksfoot, Yorkshire fog, sweet vernal, and clover (Ross et al., 1999). The second site was in 9-year-old pine forest that had been planted in 1997 following clear felling of the first-rotation forest. The pasture soil is dominated by Type I methanotrophs, while Type II methanotrophs dominate the forest soil (Singh et al., 2007). At both sites, samples were collected from the top 10 cm of the soil profile. These pasture and forest soils have bulk densities of 0.53 and 0.49 Mg m^{-3}, respectively (Tate et al., 2007). In the laboratory the samples were sieved to remove roots and other plant material, and stored at 4–6ºC for 5–6 months before the experiment began.

Methane oxidation measurements

The laboratory gas analysis system consisted of a gas mixing system where air with different CH_4 concentrations could be generated, a series of chambers containing soil samples through which the mixed gas flowed at a known rate, and a gas analysis system where the CH_4 concentration of gas entering and leaving the soil chambers could be measured. The gas mixing system consisted of two electronic gas mass flow controllers (FMA-3204, 0–500 ml min^{-1} and FMA-3205, 0–5000 ml min^{-1}, Omega Engineering, Manchester, UK). One mass flow controller was connected to a gas cylinder containing either 500 ppm CH_4 in air or 5% CH_4 in air, depending on the desired final CH_4 concentration range. The other mass flow controller was connected to a filtered compressed air supply. By altering the ratio of flow between the two mass flow controllers, air with CH_4 concentrations between ambient (ca 1.8 ppm) and several thousand ppm could be generated. The soil chambers were plexiglass cylinders with end caps sealed by O-rings. Attached to each chamber was a rotameter (FL-3802G, Omega Engineering, Manchester, UK) that controlled the volumetric flow of air through the chamber. Sub-samples of field-moist soil (equivalent to 338 g of oven-dry weight) from each site were weighed into plastic pots (650 ml) and sufficient water was added to bring the soil water content to 60% of water holding capacity. The pots were

suspended inside the chambers and held in place by a foam seal that ensured all gas entering the chamber flowed through the soil sample. Gas flowed through the chambers continuously at a flow rate of about 150 ml min^{-1}. The CH_4 and N_2O concentrations of gas entering and leaving the soil chambers were measured by a gas chromatograph (GC) (CP-3800, Varian Inc., USA) using a Flame Ionisation Detector (FID) and an Electron Capture Detector (ECD), respectively. Gas samples were drawn sequentially from each chamber outlet into the GC through a 12-port switching valve using a small vacuum pump. Before and after each soil chamber outlet measurement, a sample of gas was drawn from an empty chamber and analysed for inlet CH_4 and N_2O concentrations. The GC was periodically calibrated against gas standards of known CH_4 and N_2O concentrations spanning the range of concentrations used in the experiment.

The rate of methane oxidation (O_m, µg CH_4 kg^{-1} soil h^{-1}) was calculated as:

$$O_m = \frac{(M_{out} - M_{in})F}{S},$$

where M_{in} and M_{out} are the CH_4 concentrations of chamber inlet and outlet gas streams (µg CH_4 ml^{-1}), F is the volumetric flow rate (ml h^{-1}) and S is the mass of soil samples (kg). The rate of N_2O exchange was calculated similarly, but substituting N_2O concentrations for CH_4. The CH_4 removal efficiency (R_e, %) was calculated as:

$$R_e = \frac{(M_{in} - M_{out})}{M_{in}}100$$

Every 7 to 10 days the pots were removed from the soil chambers and weighed to determine water lost by evaporation into the chamber gas stream, and sufficient water was added to return the soil samples to 60% of water holding capacity.

Measurement protocol

Two replicates of each soil were placed in individual soil chambers and exposed to ambient CH_4 concentration (ca 1.8 ppm) for four days. The inlet CH_4 concentration was then increased in steps to 36 ppm, 65 ppm, and about 550 ppm, with soils exposed to each concentration for at least 4 days. M_{in} was then returned to ambient concentration for the final 4 days of the experiment. After each change in CH_4 concentration, chambers were allowed to equilibrate for at least 20 hours, and rates of methane oxidation were measured once per day thereafter.

3.3 Results

The experiment began on 22 May 2008. During the first 4 days' exposure to ambient CH_4 concentration (Fig. 3), O_m of the forest soil was about 5.5 µg CH_4 kg^{-1} soil h^{-1} (Fig. 4), whereas for the pasture soil O_m was about 0.7 µg CH_4 kg^{-1} soil h^{-1}, approximately 7 times lower (Fig. 4). The forest soil removed about 20% of the CH_4 from the chamber gas stream, while the pasture soil removed just over 2% (Fig. 5). There was no difference in O_m or R_e between either replicate of pasture or forest soils, and O_m and R_e remained relatively constant in both forest and pasture soils over the four-day period of exposure.

During days 5–8, M_{in} was increased approximately 18-fold to 36 ppm (Fig. 3). O_m increased

instantaneously by 6-fold and 17-fold in forest and pasture soils, to around 37 µg CH_4 kg^{-1} soil h^{-1} and 13 µg CH_4 kg^{-1} soil h^{-1}, respectively (Fig. 4). On the third day of exposure to 36 ppm CH_4, O_m declined slightly but recovered following re-watering of the soil samples, indicating a possible moisture limitation of CH_4 oxidation. R_e declined sharply in the forest soil to around 6%, but remained constant in the pasture soil at around 2% (Fig. 5).

During days 9–12, M_{in} was increased to about 65 ppm (Fig. 3). O_m again increased instantaneously in both forest and pasture soils to around 45 µg CH_4 kg^{-1} soil h^{-1} and 23 µg CH_4 kg^{-1} soil h^{-1}, respectively (Fig. 4). In the forest soil, the increase in O_m (1.2-fold) was less than the proportional increase in M_{in}. In contrast, the increase in O_m of the pasture soil (1.8-fold) was in proportion to the increase in M_{in}. R_e remained relatively constant in the pasture soil (2.1%) in response to the increase in M_{in}, but declined further in the forest soil (4.4%) (Fig. 5).

After 12 days of exposure to low or moderate CH_4 concentrations, M_{in} was increased by 8.5-fold from 65 ppm to about 550 ppm during days 13–23 (Fig. 3). In the forest soil, O_m increased by 5.2-fold to around 238 µg CH_4 kg^{-1} soil h^{-1} (Fig. 4). In the pasture soil, O_m increased by 8.4-fold to 193 µg CH_4 kg^{-1} soil h^{-1}, which was proportionally the same as the increase in M_{in}. R_e halved in the forest soil to around 2.2%, but remained relatively constant in the pasture soil at around 2.0% (Fig. 5).

Following the return to ambient CH_4 concentration on day 24 (Fig. 3), O_m decreased in both forest and pasture soils to rates almost identical to those measured at the beginning of the experiment (Fig. 4). O_m in the forest soil was about 5.7 µg CH_4 kg^{-1} soil h^{-1}, while in the pasture soil *O*m was around 0.7 µg CH_4 kg^{-1} soil h^{-1}, approximately 7-fold less. This difference is reflected in R_e, which increased in the forest soil to around 18.5% but remained relatively constant in the pasture soil at around 2.7% (Fig. 5).

During the entire experiment, there was no detectable emission or removal of N_2O from either the forest soil or the pasture soil (Fig. 6). Almost all the flux measurements fell within the range -250 to 250 ng N_2O kg^{-1} soil h^{-1}, which essentially reflects the limit of detection of the ECD detector for N_2O gas (approximately 13 ppb). In other words, if the N_2O concentration difference between chamber inlet and outlet was less than 13 ppb, then the corresponding flux rate could not be distinguished from zero.

3.4 Discussion

Methanotrophic bacteria are unique in their ability to utilise CH_4 as a sole carbon and energy source. They are ubiquitous—found in samples from muds, swamps, rivers, rice paddies, oceans, ponds, pasture and forest soils, sewage sludge—and contribute significantly to global CH_4 oxidation. The defining characteristic of all methanotrophic bacteria is the oxidation of CH_4 to methanol, catalysed by methane monooxygenase (MMO) enzymes, followed by transformation to formaldehyde. About half the formaldehyde is further oxidised to formate, and then to CO_2 yielding much of the reducing power that is needed for CH_4 metabolism (Hanson and Hanson 1996). The remainder is assimilated and used for synthesis of new cellular material. Type I and II menthanotrophs are distinguished by the metabolic pathway used to assimilate formaldehyde. In type I methanotrophs, the formaldehyde is assimilated via the ribulose monophosphate (RuMP) pathway, while in type II methanotrophs the formaldehyde is assimilated via the serine pathway. Although both type I and type II methanotrophs can be found in any aerobic environment where there is a source of CH_4, there

are certain conditions that favour growth of one type over the other. The concentrations of CH_4, oxygen and combined nitrogen are the primary determinants of the type of methanotrophs present in an environment (Hanson and Hanson 1996).

It is noteworthy that the methanotroph populations survived several months of storage with minimal ill effects in plastic bags at low temperature (4–6 ºC) and ambient CH_4 concentration. When CH_4 was provided, the methanotrophs immediately recommenced oxidation. This is encouraging as it indicates these bacteria are extremely resilient and can withstand long periods without substrate, yet are able to utilise CH_4 instantly when it becomes available. This will be vital for a biofilter in an application where inlet CH_4 concentrations will vary widely over time—for example, an intermittently occupied animal house.

At ambient CH_4 concentration, the CH_4 oxidation rate of the forest soil was 7 times greater than that in pasture soil. Given that both soils were well aerated and had similar moisture contents, this difference must be largely attributable to the different methanotroph populations present—predominately type I in the pasture soil and type II in the forest soil (Singh et al., 2007). This indicates that Type II methanotrophs have a much greater affinity for CH_4 than type I methanotrophs, and this is reflected in a greater efficiency of CH_4 uptake at low concentrations. This is consistent with the thought that Type II methanotrophs generally oxidise atmospheric CH_4 rather than the usually much higher concentrations of CH_4 produced in soil (Dunfield et al., 1999).

Methanotrophs in both soils responded almost instantaneously to increases in CH_4 concentration, which indicates that bacteria present in the soils are substrate-limited and that no *de novo* microbial synthesis had occurred. However, it became apparent that CH_4 oxidation in both soils responded differently as CH_4 concentration increased. In the forest soil, the increase in CH_4 oxidation was always proportionately smaller than the increase in CH_4 concentration. This indicates the Type II methanotrophs may have become progressively saturated with respect to substrate concentration, which is reflected by the decrease in uptake efficiency as CH_4 concentration increased. In contrast, the increase in CH_4 oxidation of the pasture soil was almost always proportional to the increase in CH_4 concentration. There was no evidence of substrate saturation, which was reflected in constant uptake efficiency over the range of measured CH_4 concentrations.

At the highest CH_4 concentration, the CH_4 oxidation rates and uptake efficiencies of both soils had converged. This suggests the forest soil may contain type I as well as type II methanotrophs, while type I methanotrophs predominate in the pasture soil. The greater CH_4 oxidation rate of forest soils over pasture soils at low CH_4 concentrations is caused by the dominance of type II methanotrophs in the forest soil, while the similar CH_4 oxidation rates at high CH_4 concentrations may result from the presence of type I methanotrophs in both soils. In contrast to type II methanotrophs, type I methanotrophs are thought to utilise sources of CH_4 within the soil that are at much higher concentrations than ambient. Clearly, in developing a CH_4 biofilter it will be critical to identify the type of methanotroph in the filter inoculum. Furthermore, these results reveal the intriguing possibility of customising CH_4 biofilters for different applications (e.g., high flow, low concentration cf. low flow, high concentration) by specifying particular methanotroph populations.

After 14 days at elevated CH_4 concentration, there was no evidence the methanotroph population had grown in either soil. When the CH_4 concentration was returned to ambient

level, the CH_4 oxidation rates of both forest and pasture soils returned to almost identical rates to those recorded at the beginning of the experiment. It is possible that longer exposure time or exposure to higher CH_4 concentrations is required to initiate growth in methanotroph populations. This may also explain why the observed CH_4 removal efficiencies were relatively low (range 2.5–20%) compared to those reported for landfill and effluent biofilters exposed to very high CH_4 concentrations (up to 85%). Melse and van der Werf (2005) showed that CH_4 removal efficiency in a pilot-scale effluent biofilter increased over 2 weeks to stabilise at 80–85% after exposure to >1000 ppm CH_4.

Finally, this experiment has provided a robust test of our new laboratory analytical system. The system is unique in comprising multiple chambers that can be automatically sampled for CH_4, N_2O, and CO_2 (not reported here) with the ability to resolve extremely low fluxes. The system will be a critical component of continued research into development of a methane biofilter.

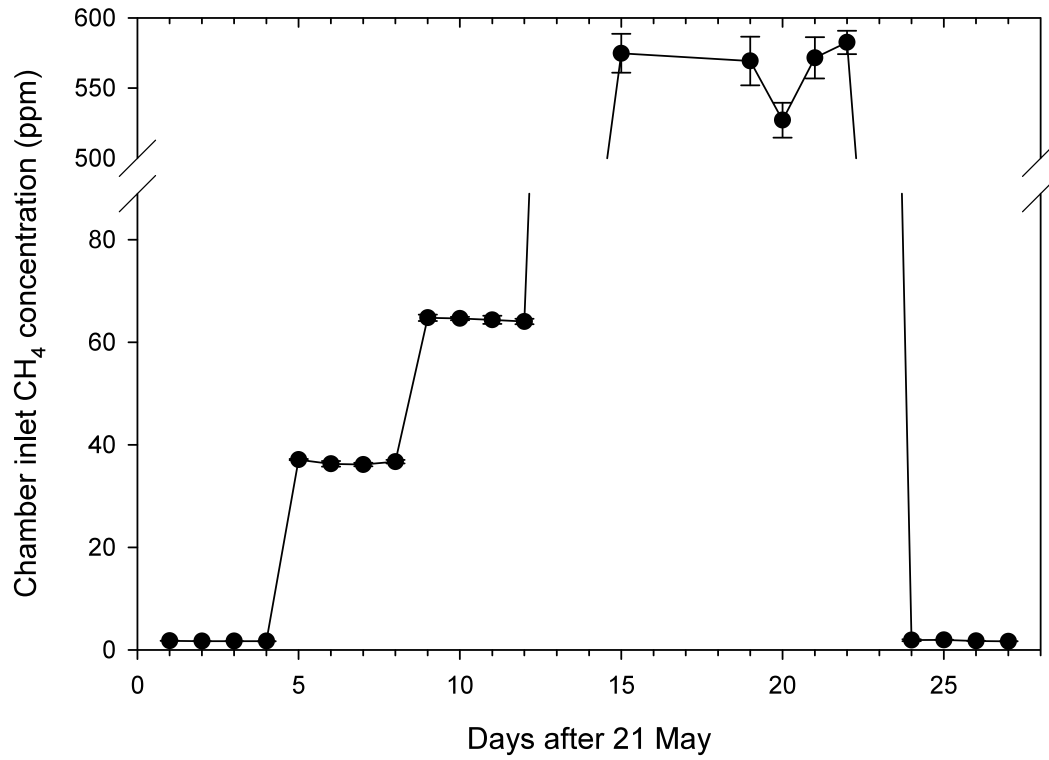

Fig. 3 Methane concentration of gas entering the soil chambers.

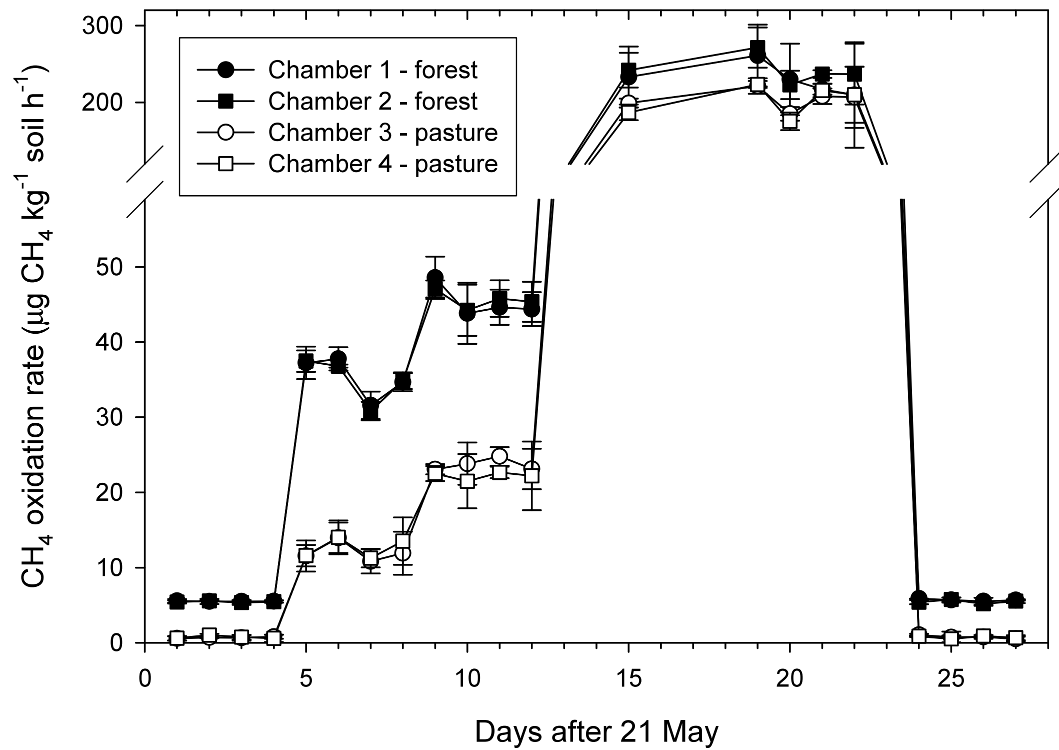

Fig. 4 Methane oxidation rate of forest (closed symbols) and pasture (open symbols) soil samples.

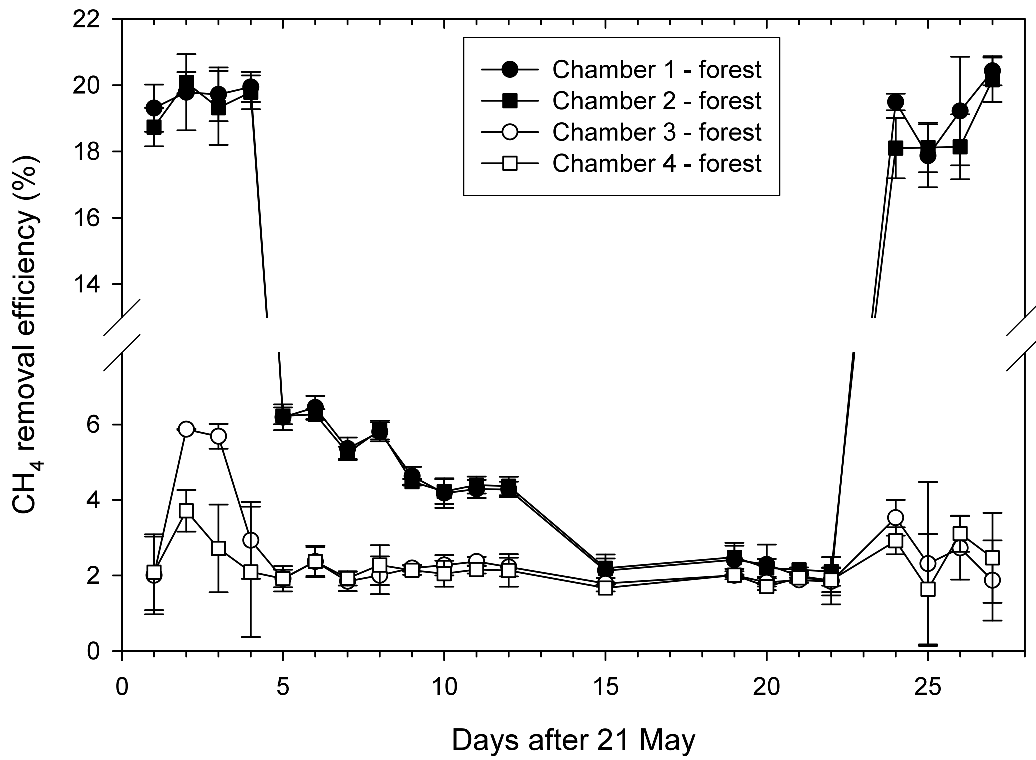

Fig. 5 Methane removal efficiency, representing the percentage of methane in the inlet gas that was oxidised, in the forest and pasture soil samples.

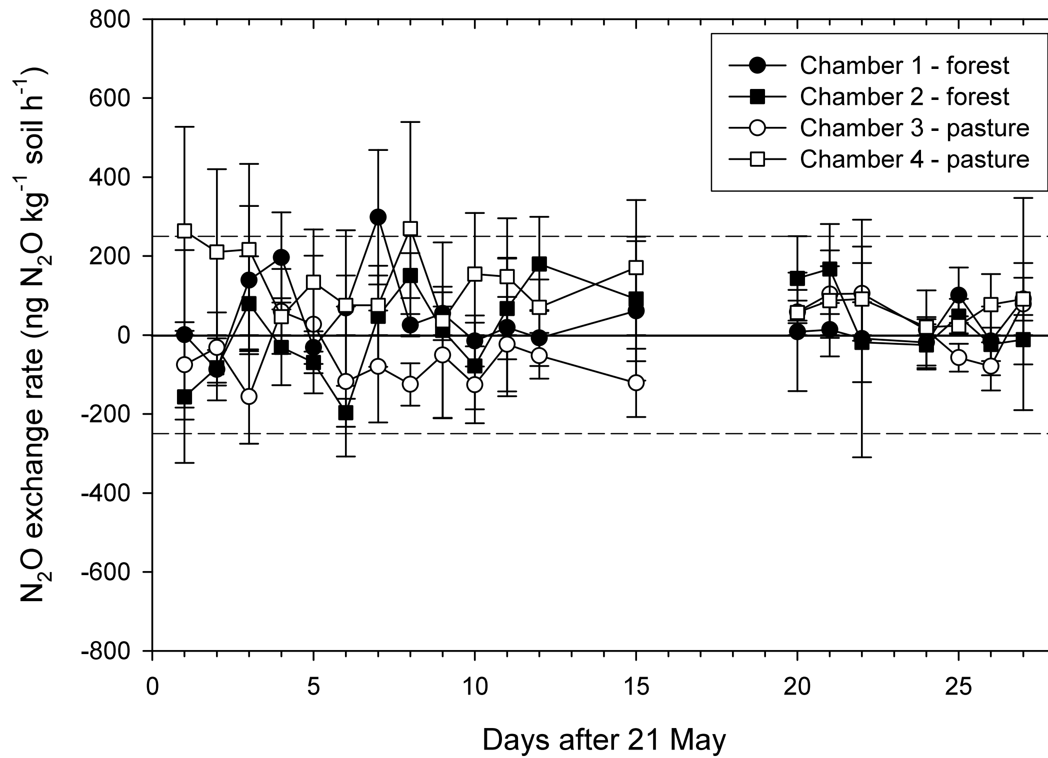

Fig. 6 The rate of nitrous oxide removal (positive values) or emission (negative values) of forest and pasture soil samples. Between days 15 and 20 the source of chamber inlet air was changed to one containing no measurable N_2O. There was also no measurable N_2O in the outlet air so calculated fluxes were zero. Dashed lines indicate the minimum flux that can be distinguished from zero given the detection limit of the GC-ECD detector.

4. Conclusions and Implications

Methane oxidation by soil methanotrophs clearly has the potential to offset increases in CH_4 emissions since 1990. In the short to medium term, revegetation of marginal pastoral hill country by shrubland could potentially offset 2–7% of the increase in CH_4 emissions since 1990. In the longer term, CH_4 oxidation rates are likely to increase further with successional development of shrubland to indigenous forest. However, CH_4 oxidation rates during the transition from pasture to shrubland are variable, and further measurements are required to understand the processes involved in this transition phase and thereby reduce measurement uncertainties.

Methanotroph biofilters that can oxidise 60–90% of the CH_4 loading have been successfully developed and tested (Nikiema et al., 2007). This technology could be applied in New Zealand to cost-effectively treat CH_4 emissions from effluent ponds on dairy farms and from landfills where the large capital expenditure required to capture and use the CH_4 for power generation is not justified, or the CH_4 concentration is below that required for combustion. If 50% of the CH_4 emitted from all animal waste effluent ponds (currently 0.8 Mt CO_2-e y^{-1}) could be oxidised in CH_4 biofilters, this would offset around 22% of the 1.8 Mt CO_2-e y^{-1} increase in national CH_4 emissions between 1990 and 2005.

Further, there is the potential to apply biofilter technology to oxidise CH_4 emitted from ruminant animals when they are concentrated in small, enclosed or partly-enclosed areas such as wintering barns or “herd homes”. We have established that soils from different land-use types differ markedly in methane oxidation rates and reaction kinetics, probably because they support different methanotrophs. Further research is required to identify optimal conditions for CH_4 oxidation in a biofilter, and to establish whether development of a CH_4 biofilter for application to housed animals is technically possible and economically feasible. Equally vital will be the engagement of farmers and manufacturers of animal housing systems to ensure this biofilter research is fully tested and applied so its potential can be realised.

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