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DAIRY HOUSING METHANE MITIGATION USING PASTURE SOILS

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Introduction

Methane (CH_4) emissions from New Zealand dairy farms are mainly contributed by enteric fermentation. In 2014, dairy cattle were reported to contribute 48% (13742 Kt CO_2–eq) of the total enteric fermentation emissions (MfE 2015). Further intensification of the dairy industry is expected to increase these emissions. As an effective way to reduce enteric CH_4 emissions from dairy cattle remains elusive, a range of mitigation options need to be explored to help reduce their emissions. One potential mitigation option is to pass CH_4–enriched air (~150 ppmv) collected from barns/ animal sheds through the adjacent agricultural soil primed with CH_4–eating bacteria, methanotrophs. Previous research indicated that the agriculture soils are capable of removing CH_4 concentrations between 3,000 and 20,000 ppmv, when primed with more than 3600 ppmv (Syed et al. 2016) but research on removal of low concentrations of CH_4 (~150 ppmv found in the housed ventilated barns) by soil was not explored.

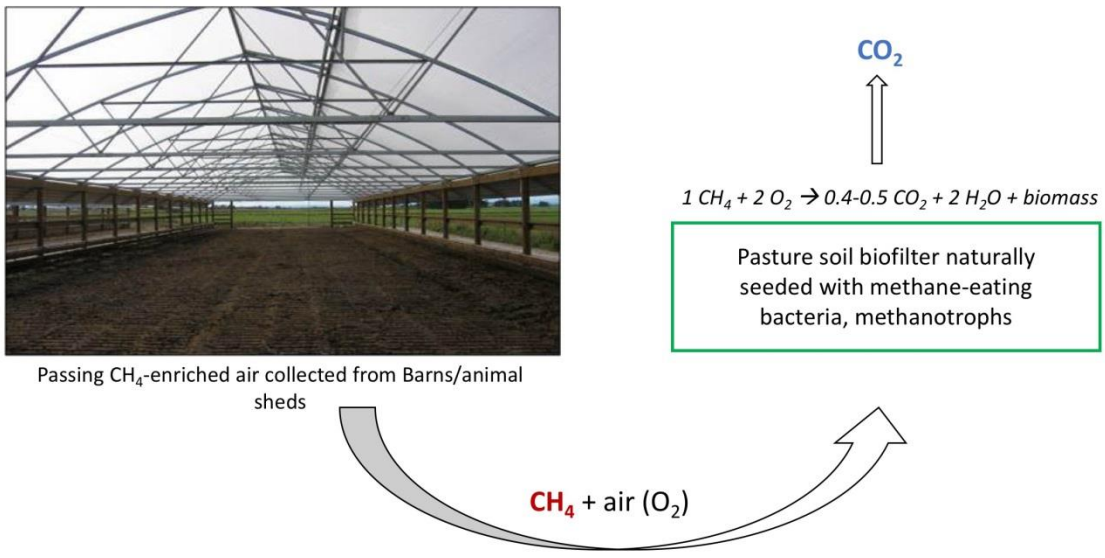

Figure 1. A potential mitigation option to reduce enteric CH4 emissions is to pass the ambient air from barns/animal sheds through a soil biofilter.

Initial research on removal of low CH_4 concentrations suggested the inability of the unprimed soil to remove CH_4 (data not shown). We anticipated that this could be due to lower abundance of methanotrophs in the soil. Therefore, in this study our aim was to determine the threshold concentrations of CH_4 required for triggering the CH_4 removal ability of four soils from major dairying areas *viz.*, Waikato (Ruakura), Taranaki, Manawatu (Massey – dairy no.4 and no.1) with contrasting organic matter contents. These soils were primed at four different concentrations (150, 1200, 2400 and 3600 ppmv CH_4) simultaneously for a period of two months. The soils primed at 2400 and 3600 ppmv that were oxidising significant amounts of CH_4 comparatively were selected for further studies. For the next phase of experiments, these soils were then exposed to low CH_4 concentrations (150 ppmv) typical of that found in barns occupied by cows for three months. This has allowed us to assess the ability of the primed soils to sustain high CH_4 removal at these low CH_4 concentrations. The objectives of this study were to (i) find the threshold concentrations required for inducing CH_4 oxidation in soils, (2) assess whether the soils can sustain their ability to oxidise 150 ppmv concentrated CH_4 emissions and (3) explore whether abundance of any sub-group of methanotrophs is related to the low CH_4 removal ability of the soil.

Materials and Methods

Four soils with contrasting organic contents were used to assess their ability to oxidised CH_4. Soils were collected at sites listed in Table 1 and were sieved to 5 mm.

Table 1. Physico-chemical properties of the agricultural soils studied

Site name	**Soil name**	**Soil type**	**Moisture content (% WHC)**	**pH (1:2.5)**	**EC (µS/cm)**	**Total C (%)**	**Total N (%)**
Massey University Dairy No.1	Manawatu Sandy Loam	Weathered fluvial recent	41	5.2	169.3	2.47	0.27
Massey University Dairy No.4	Tokamaru silt loam	Argillic-fragic Perch-gley Pallic	61	5.5	144.4	3.02	0.32
Ruakura No. 1 Dairy Farm	Horotui Silt Loam	Typic orthic allophanic	54	5.5	231.0	4.90	0.46
Stratford Dairy Farm, Taranaki	Egmont Black Loam	Typic orthic allophanic	54	6.2	150.4	9.90	0.98

Moisture content was adjusted to 40–60 % of the water holding capacity (WHC) before initiating the experiment. About 225 g of representative samples was incubated in triplicate gas-tight AGEE jars (1.8 L) and were supplied with air (O_2) and CH_4 at the beginning of a 24-hr batch period every day during the 5-months study period. The jars were incubated at constant temperature (25 °C) through out the study period. Samples were taken once every week for a period of 5 months.

Methane was measured at 0 and 24 hrs of the fed-batch period. The difference in CH_4 concentrations indicated the amount of CH_4 oxidised (%). Gas Chromatogrpahy was used to measure CH_4 concentrations using the linear standards (0, 8, 50, 100, 250 and 500 ppmv CH_4). Carbon dioxide (CO_2) was also measured to make sure the jar was gas-tight. In addition, a higher concentration of CO_2 at the end of 24-hour batch period also indicated presence of microbial acitivity (data not shown). Statistical analysis was performed using Sigma Plot (version 12). A signficance level of 0.05 was used to compare the means of triplicate measurments.

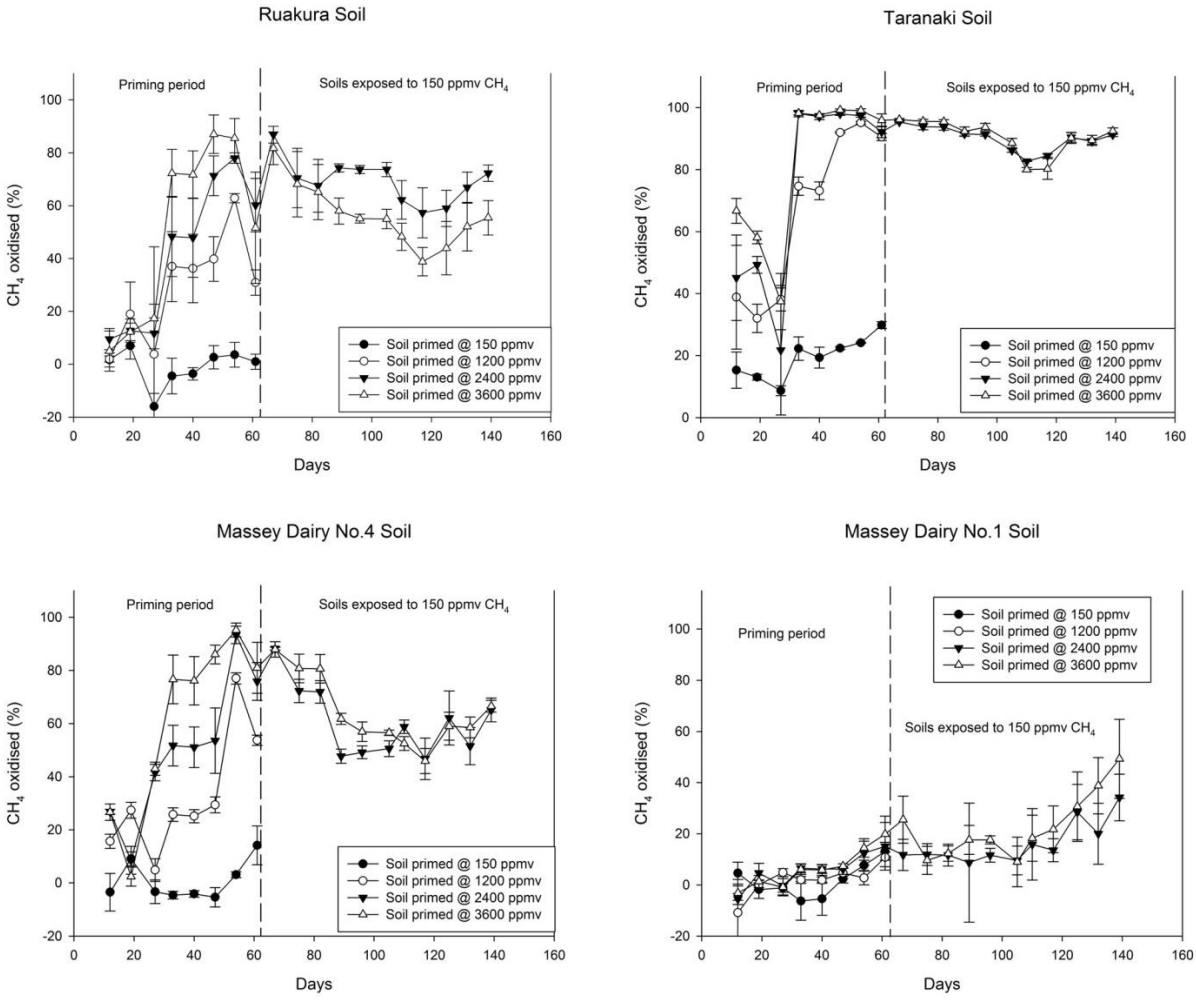

Figure 2. Methane removal (%) of all the soils over the study period.

Representative soil samples were sub-sampled on day 0, day 67 (end of two-month priming period) and the final day (end of 150 ppmv CH_4 exposure) for molecular analysis. DNA was extracted from soil samples in duplicate using a Mobio™ Powersoil DNA extraction kit (Mobio Laboratories, USA) according to the manufacturer's instructions. Primers targeting type I and type II aerobic methanotroph community (16S rRNA) and specific type II methanotroph groups like *Methylosinus* and *Methylocystis* (pmoA based) were used in this study. Standards were prepared as described in Syed et al. (2016). Reactions in duplicate were carried out in a Roche Light cycler 480^{TM} machine and were as described in Syed et al. (2016 (Lee 2008)). Melt curve analysis was done post-qPCR by acquiring fluorescence data by continuous melting of samples from 65 °C to 95 °C for 30 s. Formation of assay-specific product size was verified by gel electrophoresis on a 2.0% w/v agarose gel in 1×TBE buffer. This was stained with SyberSafe gel stain and visualized under UV light. Gene copy numbers of the samples were calculated by plotting linear regressions of crossing point (Cp) values and logarithmic gene copy number values of the standards (calculations adapted from Lee et al. (2008)). The r^2 values of the plots ranged from 0.9972 to 0.9999.

Results & Discussion

Except for Massey dairy no. 1 soil (with a total C of 2.47 %), all soils oxidised >50% CH_4 when primed with higher CH_4 concentrations of 2400 and 3600 ppmv. The higher the CH_4 priming concentration, the higher was the % CH_4 oxidised by the soils (Figure 2). For instance, Ruakura soil primed at 150, 1200, 2400 and 3600 ppmv oxidised 1, 29, 42 and 53 % CH_4, respectively. Similarly, Taranaki soil primed at 150, 1200, 2400 and 3600 ppmv oxidised 19, 67, 75, and 82 % CH_4, respectively. Interestingly, none of the soils were able to oxidise CH_4 when primed with 150 ppmv CH_4 priming concentrations. Ruakura, Taranaki, Massey dairy no.4 and 1 oxidised 1, 19.4, 0.6 and 1.7 % CH_4, respectively when primed with 150 ppmv CH_4.

Quantitative PCR analysis of the soil samples on day-0 indicated that Taranaki soil had a significantly higher abundance of both type I and II methanotrophs ($P < 0.05$), except for Ruakura soil where the type I methanotroph population was similar to that present in Taranaki soil (Fig 3). There were no significant differences in the methanotroph populations between Ruakura and Massey dairy no.4 soil. Massey dairy no.1 soil had the least abundant methanotroph population. Regardless of the soils exposed to priming concentrations of 2400 or 3600 ppmv, both type I and II methanotroph (including *Methylocystis*/*Methylosinus*) population was similar ($P > 0.05$), except for Ruakura soil when exposed to 3600 ppmv had a higher type II methanotroph population than its counterpart exposed at 2400 ppmv CH_4 ($P = 0.01$).

During the post-priming period (where the soils initially primed at 2400 ppmv and 3600 ppmv were exposed to 150 ppm for three months) – of all the soils, Taranaki (9.9 % C) consistently removed >80 % of CH_4, whereas Ruakura (4.9 % C) and Massey dairy no. 4 (3.02 % C) went through low and high CH_4 removal stages (~ 40 to 65 %). Massey dairy no.

1 soil (2.47 % C), however, removed between 9 and 30 % CH_4. This concurs with the qPCR results (Fig 3) where Taranaki soil had the highest abundance of type I, type II methanotroph (including *Methylosinus*/*Methylocystis)* population (P = 0.001–0.003). Results also indicated that the presence of type II methanotroph (including *Methylosinus*/*Methylocystis* groups) may be essential for sustaining higher oxidation rates when exposed to 150 ppmv CH_4, as seen in the Taranaki soil. This is supported by the qPCR data where Ruakura, Massey dairy no.4 and 1 soils had 48, 40 and 74 % lower abundances of type II methanotrophs than Taranaki soil by the end of the study period.

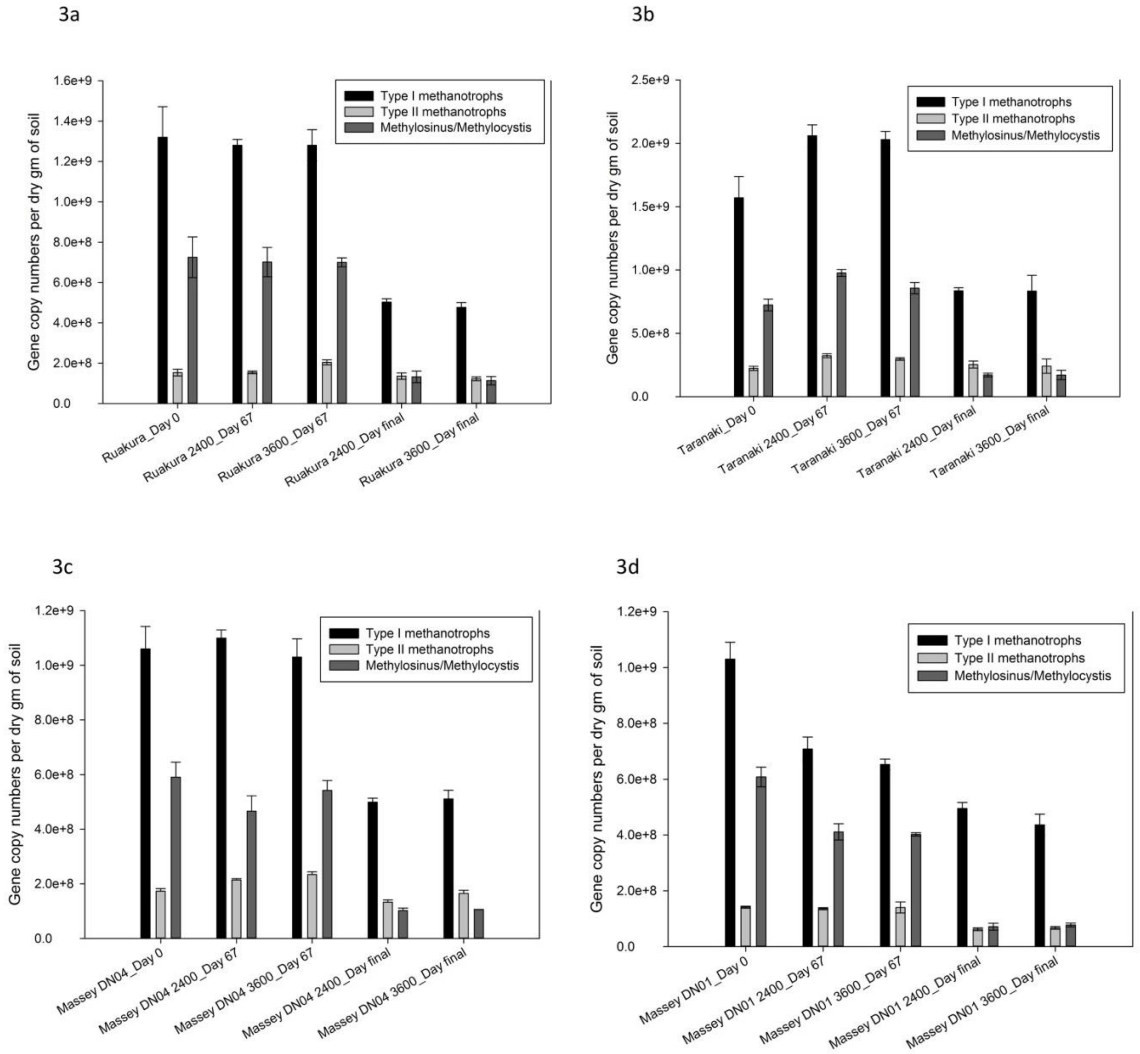

Figure 3. Type I and II methanotroph abundance in all the soils on day-0, 67 and final.. Taranaki soil supported highest growth of methanotrophs; Massey Dairy No.1 soil supported the least. Type II methanotrophs populations tended to be critical in CH_4 removal.

Results also indicated that a higher abundance of type I and II methanotrophs is not likely to enable soils to mitigate 150 ppmv CH_4, since the overall population decreased in all the soils over the study period (Fig 3). However, the presence of methanotrophs belonging to type II group may be related to CH_4 oxidation at low concentrations.

Conclusions

Of all the soils, Taranaki performed best by consistently removing >80 % of low concentrated CH_4 emissions, and Massey dairy no.1 removed least (9 – 30 %). Our results suggest that primed pasture soils high in organic matter have the potential to mitigate CH_4 emissions from housed animals. Priming of soils with higher concentrations of CH_4 (2400 or 3600 ppmv) for two months may be essential to enable the soils to remove low concentrated CH_4 (~150 ppmv observed in the ventilated barns). The outlets of the exhaust systems needed to remove hazardous ammonia produced from deposited urine in the barn will simultaneously pump the CH_4 from the sheds which can be diverted into the soils. Thus eliminating the costs to pump out CH_4.

Methanotrophs belonging to type II group may be involved in oxidising low concentrated CH_4 emissions. While further studies using RNA techniques, denaturing gradient gel electrophoresis (DGGE), terminal-restriction fragment length polymorphism (T-RFLP) and pyrosequencing over longer periods of time are likely to improve our understanding of methanotroph population dynamics in low concentrated CH_4 environment. Isolation of methanotrophs (pure/mixed cultures) from the soil and their growth in bioreactors to produce large quantities of high cell density cultures may provide an avenue for priming soils with methanotrophs and for establishing a soil biofilter in other alluvial soils low in organic matter such as Massey dairy no. 1 soil used in the current study.

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