CHARACTERISING A FIELD METHANE OXIDATION BIOFILTER – TREATING FARM METHANE EMISSIONS FROM MASSEY UNIVERSITY NO. 4 DAIRY POND

Rashad Syed [a, b], Surinder Saggar [a, b], Kevin Tate [b] & Bernd H.A. Rehm [c]

[a]Soil & Earth Sciences Group, Institute of Agriculture & Environment, Massey University, Palmerston North 4442, New Zealand
[b]Ecosystems & Global Change, Landcare Research, Palmerston North 4442, New Zealand
[c]Institute of Fundamental Sciences, MacDiarmid Institute of Advanced Materials and Nanotechnology, Massey University, Palmerston North, New Zealand
Email: syedr@landcareresearch.co.nz

Abstract
This study builds on the findings of Pratt *et al.* (2012b), demonstrating that the field soil biofilter operating at Massey No. 4 dairy farm pond can achieve high methane (CH_4) removal efficiency. The aim of the study was to characterise methanotroph (CH_4 - eating bacteria) abundance and diversity in the column biofilter that has been operating almost continuously for 5 years with little maintenance. The methanotroph abundance and diversity in the reconstituted biofilter were studied for 3 months using the molecular biology technique, quantitative polymerase chain reaction (qPCR). Biofilter parameters including moisture content, pH, microbial biomass carbon (MBC), and microbial biomass nitrogen (MBN) were measured. Results revealed that type I, type X, and type II communities of methanotrophs were present across the biofilter, but type X and type I were found to be dominant. *Methylocapsa* were significantly higher than type I and type II community, with *p* values of 0.075 and 0.089, respectively. Other subgroups were minor, and included *Methylococcus* and the *Methylobacter/Methylomonas/Methylomicrobium/Methylosarcina* genera belonging to type I; and *Methylosinus* and *Methylocystis* belonging to type II, as indicated by the respective gene copy numbers. Overall, the type I and type X populations of methanotrophs increased from day-0 to day-90, positively correlating with the increase in CH_4 removal. The maximum CH_4 removal rate achieved at the end of 90 days of study was 30.3 g m^{-3} h^{-1}, which is higher than earlier reported by Pratt *et al.* (2012b). This study demonstrated the importance of biofilter moisture content and pH in controlling CH_4 oxidation rates; and the effect of the acidic environment on changing active / inactive population dynamics of methanotrophs.

Introduction
The potent greenhouse gas (GHG) methane (CH_4) is produced by the anaerobic decomposition of manure when stored in anaerobic lagoons, liquid systems or pits. Emissions of CH_4 from New Zealand dairy effluent ponds are not accurately known (Chung *et al.*, 2013), but they contribute up to 10% of total on-farm CH_4 emissions. Manure deposited on the milking sheds, feeding pads, and stand-off pads is washed into the storage/effluent ponds. Due to the high organic C content and low oxygen/anaerobic environment in the effluent ponds, CH_4 produced by methanogens was a major component of the biogas. The amount of CH_4 produced depends on the amount, quality (volatile solids), and duration of effluent stored in the pond. When left untreated, CH_4 emissions from New Zealand dairy effluent ponds escape to the atmosphere, contributing to the greenhouse gas effect.

Conventional mitigation options available include CH_4 gas capture for power generation and flaring. Flaring can only be carried out when the CH_4 concentration is between 5 and 15 % (v/v) (Haubrichs and Widmann, 2006) but it can pose a fire hazard and, if not combusted properly, could produce toxic gases like carbon monoxide (CO) and dioxins. Capturing CH_4 for power generation involves collection of biogas and then combusting it to produce energy. Haubrichs and Widmann (2006); Menard *et al.* (2012) both report that CH_4 concentrations higher than 40% (v/v) and with minimum flow rates of 30–50 m^{-3} h^{-1} are required for power generation. This technology is economical where high flow rates and concentrations of CH_4 are produced all year round. The average herd size of NZ dairy farms is 300 and 500 in North Island and South Island, respectively. Craggs and Heubeck (2014) reported 9.8 and 6.5 kg CH_4 cow^{-1} y^{-1} from a typical dairy farm storage pond in Northland and Southland regions. According to these values, an average sized farm in North and South Islands (with 300 and 500 cows) will potentially produce 12 and 13.2 m^3 d^{-1} CH_4 respectively; whereas a farm with 1000 animals will produce 40 and 26 m^3 d^{-1} CH_4 respectively. These values suggest that power generation could not be economically feasible from New Zealand dairy effluent ponds as CH_4 flow rates of 720–1200 m^3 d^{-1} is required for continuous power generation. Mitigation of CH_4 using biofilter technology could offer the best economical option to treat emissions for an average-sized dairy farm where variable rates and concentrations of CH_4 are produced. Methane mitigation using biofilters could therefore be used to treat CH_4 emissions from sources where energy capture and flaring are not possible; for example, from small-scale dairy ponds or piggeries, barns or animal sheds, diluted coal mine CH_4 gases, wetlands, new and open landfills, petroleum industries exhaust that cannot be flared, and from solid manure storages.

Methanotrophs (CH_4 eating bacteria) form the engine of this biofilter technology. They are a diverse group of aerobic bacteria that are present naturally in all soils where CH_4 is produced. During unsuitable growth conditions, they mostly form resting stages or spores, making them very resilient and able to become active again when conditions become favourable. Methanotrophs are typically divided as type I, type II, and type X based on physiological, biochemical, and morphological properties. Type I methanotrophs are more diverse and include subgroups, viz., *Methylobacter, Methylomonas, Methylosarcina, Methylococcus, Methylomicrobium, Methylosphaera, Methylocaldum*, and unclassified *Methylococcales*. On the other hand, *Methylocystis*, *Methylosinus*, and *Methylocella* form the subgroup under type II methanotrophs. The third group, type X, which includes acidophilic methanotrophs *Methylocapsa*, possess few characteristics of both type I and type II methanotrophs. Sometimes they are included in type I or type II, but their classification is not clear (Hanson and Hanson, 1996). The growth and activity of these different subgroups of methanotrophs are optimal under different abiotic and biotic conditions (Knief *et al.*, 2003; Chang *et al.*, 2010; Henneberger *et al.*, 2011; Ruo *et al.*, 2012). Knowledge of the characteristics of these sub-groups is needed so that these organisms can be efficiently utilised to mitigate CH_4 emissions. This study was therefore undertaken to help develop an efficient biofiltration system by encouraging growth and activity of the dominant active methanotrophs.

Biofiltration study carried out by Pratt *et al.* (2012b) demonstrated high CH_4 removals up to 16 g m^{-3} h^{-1} from a field column biofilter operating at Massey No.4 dairy pond, Palmerston North. The focus of our current research is on the engine of the biofilter; in particular how methanotroph abundance and diversity have influenced biofilter performance since it began operating about 5 years ago. The objectives of this research are to (1) study the importance of moisture factor controlling CH_4 oxidation in the biofilter – whether reconstitution of biofilter material (with 60% WHC) could allow the biofilter to regain its efficiency; (2) determine the

ability of the biofilter to perform at lower pH; (3) characterise methanotrophs abundance and diversity using qPCR and identify the active groups involved in CH_4 removal.

Materials and Methods

Volcanic pumice soil, isolated from the cover soil of the Taupo landfill, New Zealand, was combined with perlite (to enhance aeration) and used as a biofilter medium. This combination demonstrated ideal physical characteristics to support CH_4 oxidation in earlier studies (Tate *et al.* (2012); (Pratt *et al.*, 2012a, 2012b; Pratt *et al.*, 2013). The material from the original biofilter was reconstituted by gentle mixing, setting the moisture content to about 60% of WHC (water holding capacity), and monitoring its performance for 90 days. Several parameters, including pH, moisture content, temperature, gene copy numbers using qPCR, microbial biomass C, and microbial biomass N, were monitored. The temperature of the biofilter (average 21^{o}C) was continuously monitored every half hour throughout the study period using an automatic data logger. Moisture content and pH were measured four times over the 90-day period. Diversity and abundance of methanotrophs were studied by extracting DNA and amplifying specific DNA regions with group specific primers. Six primer-sets targeting three main groups and three sub-groups with in type I, type X and type II methanotrophs were studied (Table 1). Microbial biomass C and N were measured to determine the increase in total microbial biomass, while qPCR was used to track changes in the methanotroph community over time. A paired *t-test* was used to compare the statistical significance of the gene copy number data among two groups.

Biofilter setup:

The field biofilter (1 m high and 0.35 m in diameter) was sited beside Massey University No. 4 dairy farm effluent pond, as previously illustrated by Pratt *et al.* (2012b). Biofilter material (58L) was composed of 50/50 (v/v) mixture of volcanic pumice soil and light weight material, perlite. Biogas (65% CH_4 (v/v), 25% CO_2 (v/v), H_2S and other unknown volatile compounds) was collected from a 4-m^2 section of the pond. About 10% of the biogas was fed to the biofilter via a flow controller that monitors the biogas flow rate and temperature of the pond and biofilter half hourly. Air was fed to biofilter at a flow rate of 1000 ml/min to keep the CH_4/O_2 ratio more than 1:3 to enhance full aerobic CH_4 oxidation. Ten sample ports, spaced 5cm apart, were present along the side of the biofilter to facilitate gas sampling at various depths in the biofilter.

Gas samples:

Gas samples were taken from the biofilter in duplicates over the course of the experimental study on day 0, day 10, day 29, and day 90. Samples from the inlet, 10 ports along the height of the biofilter, and the outlet, were collected in gas tight vials. The gas samples were analysed in the laboratory for CH_4, N_2O and CO_2 concentrations by gas chromatography (Varian CP-3800) using a flame ionisation detector (FID), thermal conductivity detector (TCD) and electron capture detector (ECD), respectively. A method file was built using the GC software by incorporating CH_4, CO_2 and N_2O standards ranging from 0 to 600 000 ppm, 0 to 200 000 ppm, and 0 to 2000 ppb concentrations respectively.

Soil samples:

Biofilter soil samples were taken along the depth of the biofilter using a core borer tool of inner diameter of 3 cm. Subsamples comprising 7-cm increments were collected in a clean plastic bag/container and stored below 4^{o}C for further analysis. The sample slots were back-filled with spare soil of the same composition from the laboratory. After thorough mixing, representative subsamples were used for determinations of moisture content, pH, microbial

biomass carbon (MBC), microbial biomass nitrogen (MBN) and DNA extraction for quantitative PCR analysis.

Moisture contents were determined by overnight oven drying at 105^{o}C. Moisture content (% dry wt) was calculated as the percentage of water loss before and after the drying. Procedures described by Vance *et al.* (1987); Cabrera and Beare (1993) were followed for MBC and MBN analysis. Before determining pH, the soil aliquots were air dried for 24 h and then mixed with de-ionised water in 1:10 ratio. The slurry was mechanically mixed and left undisturbed overnight. The next day, pH was calibrated and measured at the soil-water interphase.

DNA extraction, PCR and qPCR:
Soil aliquots from the 7-cm fractions were homogenized and extracted in duplicates using Mobio ^{TM} Powersoil DNA extraction kit (Mobio Laboratories, USA) according to the manufacturer's instructions. Briefly, 0.25 gm of soil was added to the bead tube containing proprietary buffer (supplied by the manufacturer) and vigorously vortexed for 10 minutes to break the bacterial cells. Cleaning reagents were then added according to the protocol, to extract and purify DNA. Extracted DNA was later quantified (OD at A260) using UV Spectrophotometer (Nanodrop). The purity of the DNA or protein contamination of the extracted sample was analysed by looking at the A260/A280 ratio (generally, 1.80/2.0 ratio is acceptable; all samples had a ratio of 1.88/1.95). Alternatively, extracted DNA was run on the gel electrophoresis chamber to confirm DNA quality. Clear bands with no smearing were seen, indicating the purity of the extracted DNA.

Primers used in this study are listed in Table 1. Reaction conditions were modified and standardised to suit this study. PCR was carried out in 40 uL reactions using a thermocycler (MaxyGene ^{TM}). Reaction mixtures were prepared as follows: 20 uL of one taq ^{TM} master mix (NEB, UK), 1 uL of forward primer, 1 uL of reverse primer, 2 uL of target DNA (diluted 1:25 with sterile PCR-grade water) and water was added to adjust the volume to 40 uL. The thermal profile consisted of 40 cycles of denaturation at 95^{0}C for 25 sec, annealing at assay-specific temperature (see Table 2) for 30s and elongation at 72^{0}C for 45s. PCR products were run on 2% agarose gel in TBE buffer to confirm target specific amplification by visualising band size.

Quantitative PCR standards were prepared by cloning purified assay-specific amplified genes into the E. coli host. Commercial kit (Topo ^{TM} TA, Invitrogen) was used to perform molecular cloning following manufacturer's instructions. Briefly, 6 assay-specific products were individually ligated in to the vector and transformed in to Top10 E. coli cells by heat shock. Successfully transformed cells were selected based on visual coloration of the cells (blue or white). White colonies were analysed for target gene presence using colony PCR. Successful individual colonies were cultured in small flasks and plasmids containing transformed genes of interest were harvested using a commercial kit (Gen elute ^{TM} mini prep plasmid isolation kit) following the manufacturer's instructions. M13 forward and reverse primers were used to amplify the target gene by PCR; products were purified and quantified using a spectrophotometer (Nanodrop). These genes were then serially diluted from 1/100 to 1/1000000 using sterile PCR grade water at standards of known concentrations. Each qPCR reaction volume (10 uL) consisted of 5 uL ssofast ^{TM} qPCR master mix, 0.6 uL each of forward and reverse primers, 1 uL of DNA, and sterile PCR grade water made up to final volume of 10 uL. Sample DNAs were diluted 1/25 times to reduce the effect of inhibitors in the sample. Assay-specific standards in duplicates (for calibration curve) and negative controls were run along with the samples. Reactions in duplicates were carried out in a Roche

Light cycler 480 ™ machine with the following thermal profile: initial denaturation at 94°C for 15s; 40 cycles of denaturation at 95°C for 5s; annealing at assay-specific temperature (Table 2) for 25s; and data acquisition at 82°C for 4s. Melt curve analysis was done post-qPCR by acquiring fluorescence data by continuous melting of samples from 65°C to 95°C for 30s. In addition, the amplified products from qPCR were run on 2% TBE gel to confirm formation of assay specific product size. Gene copy numbers of the samples were calculated by plotting linear regression of crossing point (C_p) 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 0.9743 to 0.9999.

Primers	Assay name	Target Group	Type	Reference
II223F / 646R *(CGT CGT ATG TGG CCG AC / CGT GCC GCG CTC GAC CAT GYG)*	Type II pmoA	*Methylosinus* group	Type II methanotroph	Kolb *et al.* (2003)
A189F / Mb601R *(GGN GAC TGG GAC TTC TGG / ACR TAG TGG TAA CCT TGY AA)*	MBAC pmoA	*Methylobacter* and *Methylosarcina* group	Type I methanotroph	Kolb *et al.* (2003)
A189F / Mc468R *(GGN GAC TGG GAC TTC TGG / GCS GTG AAC AGG TAG CTG CC)*	MCOC pmoA	*Methylococcus* group	Type I methanotroph	Kolb *et al.* (2003)
A189F / Mcap630R *(GGN GAC TGG GAC TTC TGG / CTC GAC GAT GCG GAG ATA TT)*	MCAP pmoA	*Methylocapsa*	Type II methanotroph	Kolb *et al.* (2003)
Type IF/ IR *(ATG CTT AAC ACA TGC AAG TCG AAC G / CCA CTG GTG TTC CTT CMG AT)*	Type I 16SrRNA	*Methylobacter*, *Methylosarcina*, *Methylococcus*, *Methylocaldum*, *Methylomicrobium*, *Methylomonas*, *Methylosphaera* and unclassified *Methylococcales*	Type I methanotroph	Chen *et al.* (2007)
Type IIF/ IIR *(GGG AMG ATA ATG ACG GTA CCW GGA / GTC AAR AGC TGG TAA GGT TC)*	Type II 16SrRNA	*Methylocystis*, *Methylosinus*, *Methylocella*, *Methylocapsa* and unclassified *Methylocystaceae*	Type II methanotroph	Chen *et al.* (2007)

Table 1. 16S*rRNA* and *pmoA* primers used in this study

Primer Assay	PCR annealing temperature °C	Product size (bp)	qPCR annealing temperature °C	qPCR- fluorescence data acquisition temperature °C
MBAC	54	432	58	82
MCOC	58	299	58	82
MCAP	50	461	55	82
Type II pmoA	63	444	69	82
Type I 16SrRNA	60	673	65	65
Type II 16SrRNA	60	525	65	65

Table 2. Standardised PCR and qPCR annealing temperatures, product size (bp) and data acquisition temperatures for different assays

Results & discussion:

The amount of CH_4 fed (influx) into the biofilter was maintained constant at 52 g m^{-3} h^{-1} during the 90 days period (Fig. 1). The CH_4 removal increased slowly and steadily from 40% on day-10 to 57% on day-90. There was a slight dip on day-29 (~37%), which could be accounted for by the proportion of active soil that was removed from the biofilter for sampling on day-10. While the sample slot was back-filled with fresh soil, time was required for the back-filled soil to become acclimatised and fully active in consuming CH_4. The highest CH_4 removal achieved by the biofilter at the end of the 90 day study period was 30.3 g m^{-3} h^{-1}. This is much higher than was earlier reported by Pratt *et al.* (2012b). It should be noted here that the CH_4 removal achieved 40% removal in just 10 days of CH_4 feeding, indicating that the methanotroph community is very resilient and can revive very quickly from a dormant state when sufficient substrate (CH_4) and optimal moisture conditions are available. Figure 2 shows the CH_4 flux across the vertical length of the biofilter–depth, 0 and 54 cm represent top/outlet and the bottom/inlet of the biofilter respectively. Day 0 shows no difference in the CH_4 flux between the bottom and top ends of the biofilter, indicating no CH_4 removal across the biofilter. Subsequently, the difference increased significantly over time. The lower and middles regions of the biofilter were sites of greatest oxidation compared to the top region. There are some reports of compost-based biofilters producing nitrous oxide (N_2O), which is 298 times more potent than the CO_2. While N_2O emissions from our biofilter increased slightly over time, concentrations remained close to ambient air concentrations (270 ppb).

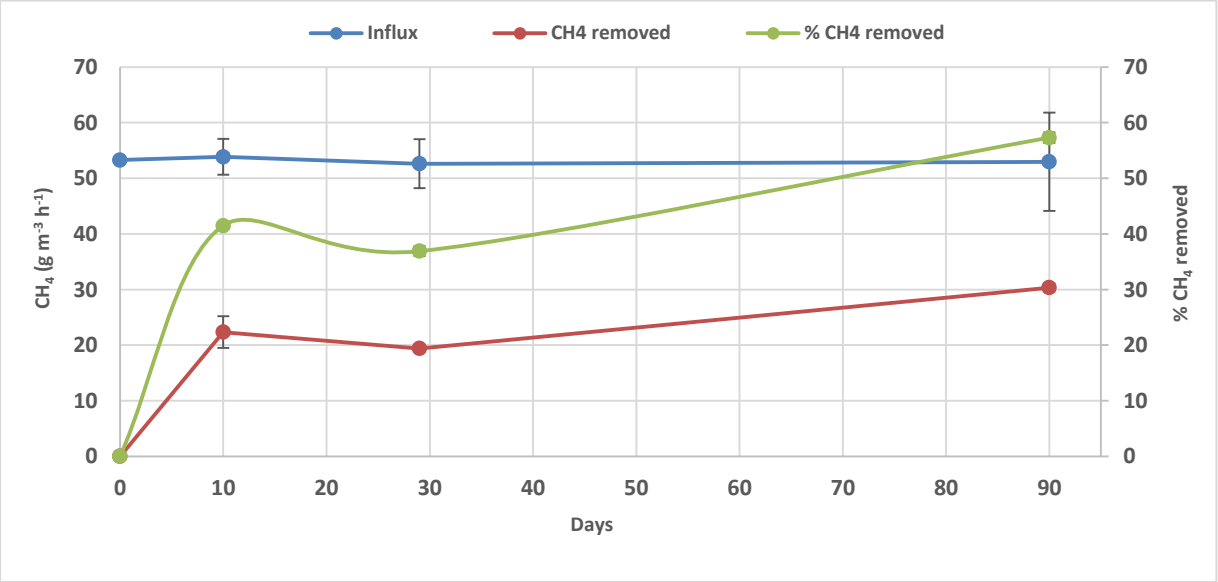

Figure 1. Methane removed by the biofilter. Error bars are the standard deviation from the mean of duplicate measurements.

Moisture content is one of the main factors controlling CH_4 oxidation. Optimal moisture content enables the transport of nutrients, and gaseous exchange to the methanotrophs. Although moisture content decreased overall from about 100 % (dry wt) at the start of study period to 80 (% dry wt) by day-90, values remained near optimal (30-70 % dry wt) during the 90-day period. The upper region in the biofilter appeared to become drier than the middle or

lower regions of the biofilter. This might be because most of the CH_4 removal occurs in the middle and lower regions of the biofilter. In addition, the presence of the high flow rate of air (1000 ml/min) tends to dry the biofilter material more than is compensated for by the H_2O produced through CH_4 oxidation.

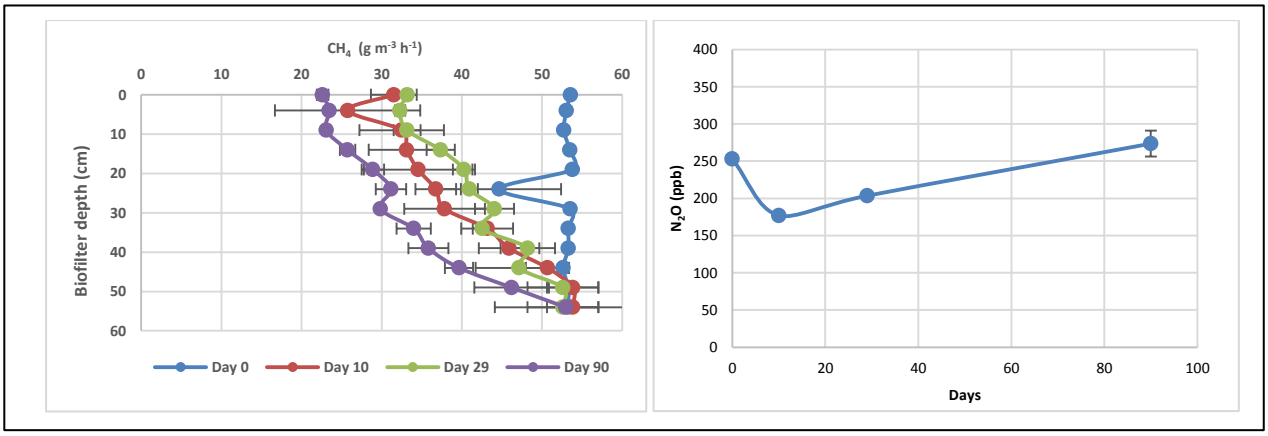

Figure 2 (a) CH_4 flux (with standard deviations) across various depths of the biofilter 0cm and 54 cm indicating the top/outlet and bottom/inlet of the biofilter, respectively **(b)** N_2O (ppb) emissions from the biofilter outlet during the study period, with standard deviations.

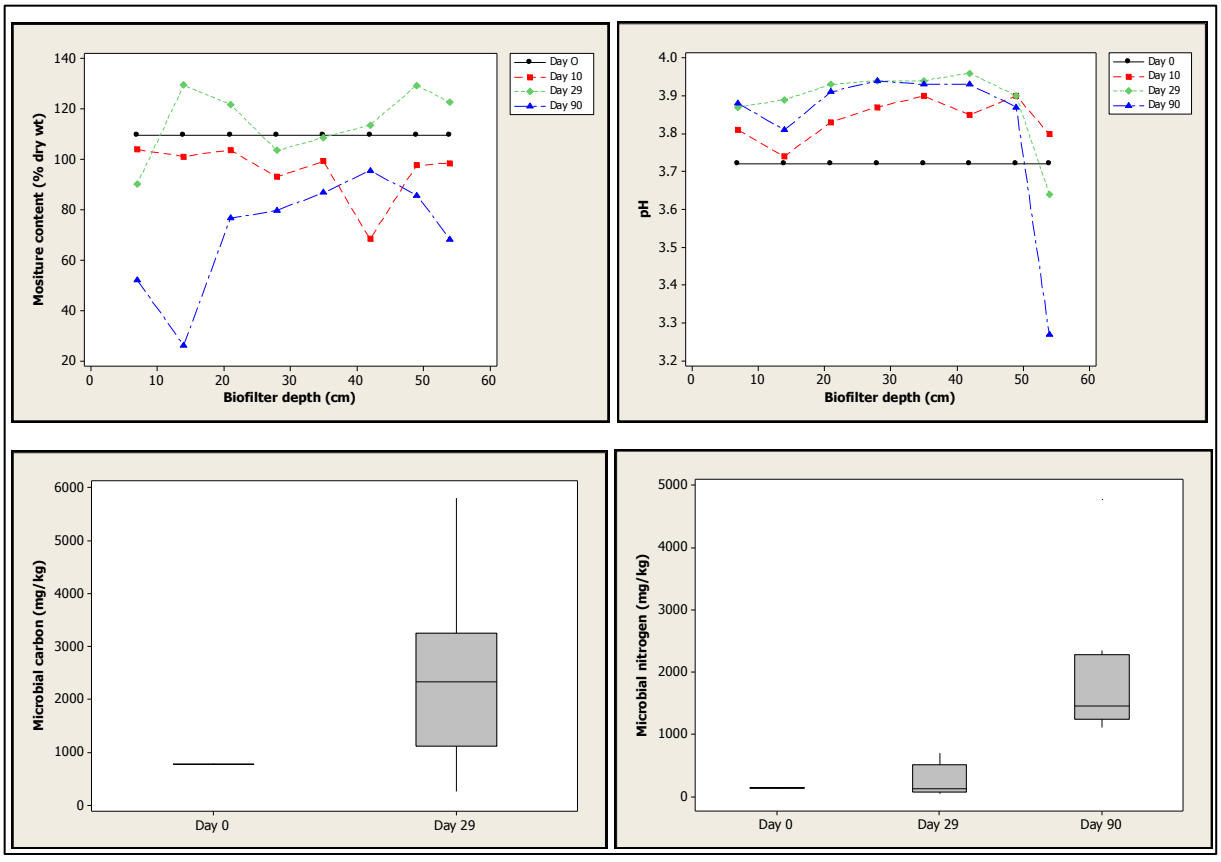

Figure 3 Moisture content, pH, MBC and MBN parameters in the biofilter

Another key abiotic parameter, pH, remained around 3.8 during the study period. Some acidification was noted at the base of the biofilter where pH dropped to 3.27 by the end of 90 days. In their previous study, Pratt *et al.* (2012b) attributed this drop in pH to the oxidation of H_2S to H_2SO_4. They also suggested that pH could be raised in the biofilter by passing biogas through lime or iron chips before feeding it in to the biofilter. The MBC and MBN results (Fig. 3) indicated an increase in microbial biomass, including methanotrophs, in the biofilter. Day-90 data for MBC are not available, but the increase in nitrogen on day-90 suggests there would also have been a proportionate increase in C content.

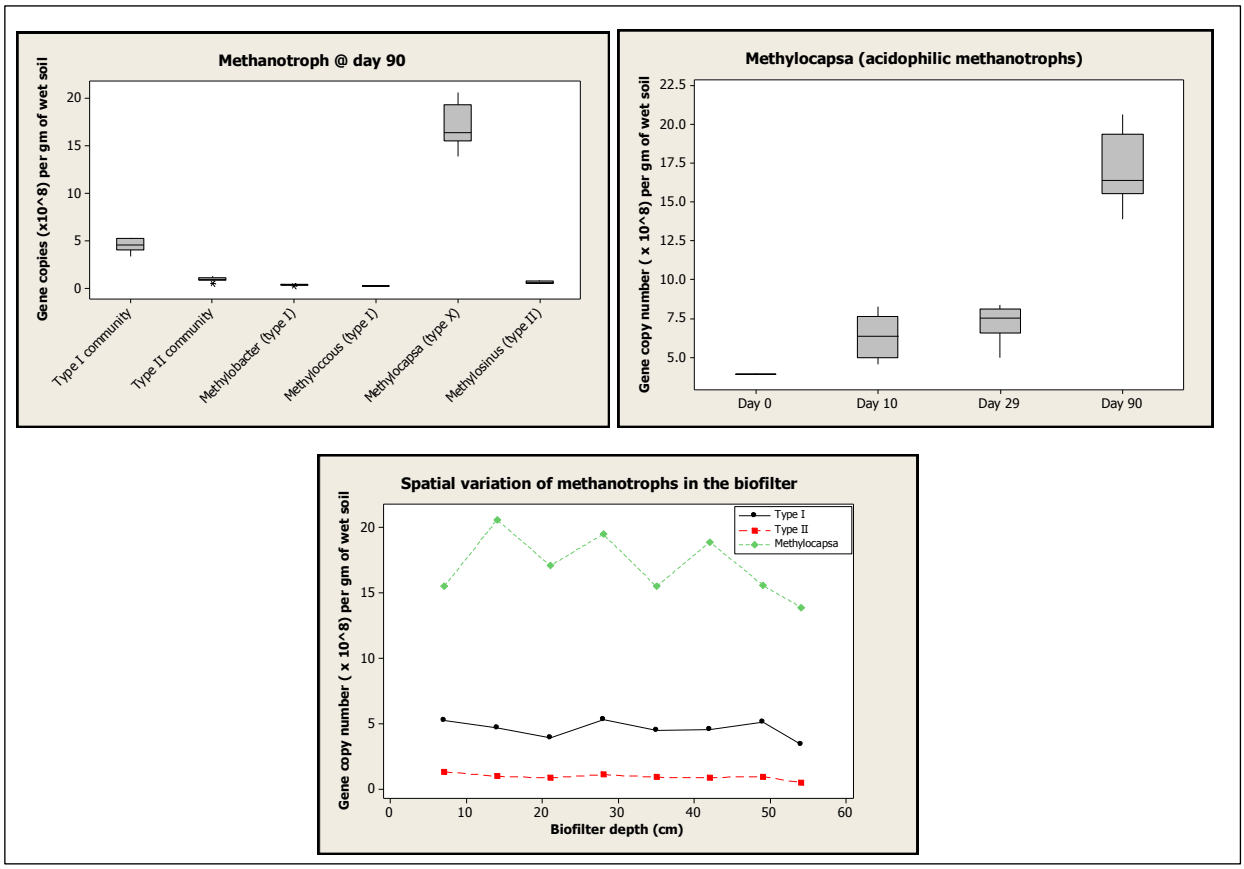

Figure 4 Plots showing a) methanotroph community at the end of study b) dominant group *Methylocapsa* population change during the study c) spatial variation of type I, type II and M*ethylocapsa* methanotrophs in the biofilter at day 90.

	Type I	Type II	pmoA type II	MCAP	MBAC	MCOC
Day 0	3.96 ± 0.16	1.91±0.05	1.06 ± 0.09	4.18 ± 0.21	0.39 ± 0.02	0.34 ± 0.02
Day 10	3.26 ±0.24	0.91±0.03	0.58 ± 0.02	6.34 ± 0.63	0.20 ± 0.0	0.43 ± 0.13
Day 29	4.10 ± 0.27	1.23±0.07	0.68 ± 0.04	7.25 ± 0.68	0.23 ± 0.0	0.33 ± 0.03
Day 90	4.58 ± 0.20	0.93±0.05	0.62 ± 0.05	17.1 ± 1.84	0.33 ± 0.02	0.25 ± 0.02

Table 3. Average gene copy number with standard deviations ($\times 10^8$) per gm of wet soil over the study period

Quantitative PCR revealed some interesting characteristics relating to methanotrophs abundance and diversity. Assay-specific primers amplified various groups of methanotrophs, indicating the presence of a diverse group of methanotrophs in our biofilter. According to gene copy numbers, the average gene copy number of the methanotrophs on day-90 was higher than that present at day-0, suggesting an increase in total methanotroph population. By day-90, methanotrophs belonging to the acidic group, *Methylocapsa,* along with the type I methanotrophs were significantly more dominant than other groups, as depicted by the gene copy numbers (Fig. 4). Overall, *Methylocapsa* gene copy numbers were significantly higher than the type I and type II communities, with *p* values of 0.075 and 0.089 respectively The type II community tends to be less dominant than the type I, which concurs with previous modelling results reported by Tate *et al.* (2012) for laboratory-based column biofilters. The presence of type 1 methanotrophs as the dominant group is not typical for landfill cover soils. However, Henneberger *et al.* (2011) reported the dominance of a type I community in their landfill cover soil. This concurs with our results that the type I community is more dominant than the type II community, with a *p* value of 0.004. Most reports in the literature suggest type I methanotrophs are dominant at higher CH_4 concentrations, and type II at lower concentrations, as for our biofilter. This suggests that when CH_4 concentrations are high (30,000–60,000 ppm), type I methanotrophs are the dominant group. With regard to presence of *Methylocapsa* in our acidic biofilter, Dedysh *et al.* (2003) found a similar group of methanotrophs belonging to *Methylocapsa* in his acidic peat soils, which indicates the ability of *Methylocapsa* to grow in acidic conditions.

Our results indicate pH was an important factor in selecting the active groups of methanotrophs- *Methylocapsa* and *type I* methanotroph, while effecting the growth of other groups viz., *Methylobacter*, *Methylococcus*, *Methylomonas* belonging to *type I;* and *Methylocystis* and *Methylosinus* belonging to *type II*. Despite the sub-groups of type I (MBAC & MCOC) being less dominant or constant during the study period, type I methanotrophs (Type I 16SrDNA primer) did seem to be increasing in gene copy numbers. This suggests that either novel species belonging to type I were detected by Type I primers, or there could be *Methylocaldum* (belonging to type I) species present in the soil that were not picked up by group-specific MBAC primers, but were identified by Type I primers. More investigations now appear warranted using primers to target the *Methylocaldum* group or using DGGE or T-RFLP to identify these methanotrophs, thereby helping to optimise biofilter performance.

Conclusion

This study provided insight into the methanotroph abundance and diversity in the biofilter using the qPCR technique. Other molecular techniques like denaturing gradient gel electrophoresis (DGGE), T-RFLP (terminal restriction fragment length polymorphism), or pyro-sequencing will be used in future to compliment these findings based on qPCR technique. In addition, DGGE and T-RFLP tools are capable of identifying novel strains in our biofilter methanotroph community. Moisture was an important factor controlling biofilter CH_4 oxidation. Reconstitution of biofilter material at 60% WHC increased the CH_4 oxidation rate, whereas no or very little oxidation was observed in the non-reconstituted or dry biofilter material (with 12.5 % WHC). The presence of active *Methylocapsa* supported the ability of the biofilters to perform at low pH. Other groups like *Methylobacter* / *Methylosinus* / *Methylococcus* were least dominant, suggesting that acidic pH was detrimental to their growth. Methanotrophs (including *Methylocapsa* and *type I* community) did not exhibit any significant spatial variability in the biofilter, indicating these organisms are not much affected

by the changing concentrations of CH_4 and O_2 with depth in the biofilter, although most CH_4 removal was taking place in the middle and lower regions of the biofilter.

By the end of 90 days, biofilter efficiency reached 60 %, offsetting 30.3 g m^{-3} h^{-1} or 1.09 m^3 m^{-3} d^{-1} CH_4, which is 47% higher than that earlier reported by Pratt *et al.* (2012b). Based on the typical CH_4 emission value of 45 m^3 d^{-1} reported by Craggs *et al.* (2008), about 50 m^3 of biofilter was required to treat all CH_4 emissions as previously reported by Pratt *et al.* (2012b). However, a biofilter of about 41 m^3 (about 20% less size) would be required to mitigate CH_4 emissions from a typical New Zealand dairy effluent pond, based on the present CH_4 removal rate. Current research aims to optimise the CH_4 removal rates, so that scaling up the technology can be achieved. Results also demonstrate that the biofilter can regain efficiency and operate with very little maintenance for a very long time with no media or chemical additions made except for adding water to keep the soil material moist enough for effective nutrient or gas transport. The biofilter reconstituted from earlier material took about 3 months to reach 60% efficiency, indicating the need to improve the start-up period of biofilter. This can best be achieved by studying various physical and chemical parameters and their effect on methanotroph abundance and diversity to develop an efficient biofiltration system.

References:

Cabrera, M.L., Beare, M.H., 1993. Alkaline Persulfate Oxidation for Determining Total Nitrogen in Microbial Biomass Extracts. American Society of Agronomy 57, 1007-1012.

Chang, C.-Y., Tung, H.-H., Tseng, I.-C., Wu, J.-H., Liu, Y.-F., Lin, H.-M., 2010. Dynamics of methanotrophic communities in tropical alkaline landfill upland soil. Applied Soil Ecology 46, 192-199.

Chen, Y., Dumont, M.G., Cébron, A., Murrell, J.C., 2007. Identification of active methanotrophs in a landfill cover soil through detection of expression of 16S rRNA and functional genes. Environmental Microbiology 9, 2855-2869.

Chung, M.L., Shilton, A.N., Guieysse, B., Pratt, C., 2013. Questioning the Accuracy of Greenhouse Gas Accounting from Agricultural Waste: A Case Study. Journal of Environmental Quality.

Craggs, R., Heubeck, S., 2014. Methane capture from dairy farm ponds. Biofilter's workshop. NIWA, New Zealand.

Craggs, R., Park, J., Heubeck, S., 2008. Methane emissions from anaerobic ponds on a piggery and a dairy farm in New Zealand. Australian Journal of Experimental Agriculture 48, 142-146.

Dedysh, S.N., Dunfeld, P.F., Derakshani, M., Stubner, S., Heyer, J., Liesack, W., 2003. Di¡erential detection of type II methanotrophic bacteria in acidic peatlands using newly developed 16S rRNA-targeted fuorescent oligonucleotide probes. FEMS Microbial Ecology, 299-308.

Hanson, R.S., Hanson, T.E., 1996. Methanotrophic Bacteria. Microbiological Reviews 60, 439-471.

Haubrichs, R., Widmann, R., 2006. Evaluation of aerated biofilter systems for microbial methane oxidation of poor landfill gas. Waste Management 26, 408-416.

Henneberger, R., ke, C.L., Mosberger, L., Schroth, M.H., 2011. Structure and function of methanotrophic communities in a landfill-cover soil. FEMS Microbial Ecology, 52-65.

Knief, C., Lipski, A., Dunfield, P.F., 2003. Diversity and Activity of Methanotrophic Bacteria in Different Upland Soils. Applied and Environmental Microbiology 69, 6703.

Kolb, S., Knief, C., Stubner, S., Conrad, R., 2003. Quantitative Detection of Methanotrophs in Soil by Novel pmoA-Targeted Real-Time PCR Assays. Applied and Environmental Microbiology 69, 2423-2439.

Lee, C., Lee, S., Shin, S.G., Hwang, S., 2008. Real-time PCR determination of rRNA gene copy number: absolute and relative quantification assays with Escherichia coli. Applied Microbial Bioetchnology 78, 371-376.

Menard, C., Ramirez, A.A., Nikiema, J., Heitz, M., 2012. Biofiltration of methane and trace gases from landfills: A review. Environmental reviews 20, 40-53.

Pratt, C., Walcroft, A.S., Deslippe, J., Tate, K.R., 2013. CH_4/CO_2 ratios indicate highly efficient methane oxidation by a pumice landfill cover-soil. Waste Management 33, 412-419.

Pratt, C., Walcroft, A.S., Tate, K.R., Ross, D.J., Roy, R., Reid, M.H., Veiga, P.W., 2012a. In Vitro Methane Removal by Volcanic Pumice Soil Biofilter Columns over One Year. Journal of Environmental Quality 41, 80-87.

Pratt, C., Walcroft, A.S., Tate, K.R., Ross, D.J., Roy, R., Reid, M.H., Veiga, P.W., 2012b. Biofiltration of methane emissions from a dairy farm effluent pond. Agriculture, Ecosystems and Environment 33-39.

Ruo, H., Wooller, M.J., Pohlman, J.W., Quensen, J., Tiedje, J.M., Leighb, M.B., 2012. Shifts in Identity and Activity of Methanotrophs in Arctic Lake Sediments in Response to Temperature Changes. Applied and Environmental Microbiology 78.

Tate, K.R., Walcroft, A.S., Pratt, C., 2012. Varying atmospheric methane concentrations affect soil methane oxidation rates and methanotroph populations in pasture, an adjacent pine forest, and a landfill. Soil bology and biochemistry, 75-81.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19, 703-707.