Global Change Biology

Global Change Biology (2015) **21**, 3864–3879, doi: 10.1111/gcb.12974

Unexpected stimulation of soil methane uptake as emergent property of agricultural soils following bio-based residue application

ADRIAN HO[1], ANDREAS REIM[2], SANG YOON KIM[1], MARION MEIMA-FRANKE[1], AAD TERMORSHUIZEN[3], WIETSE DE BOER[1], WIM H. VAN DER PUTTEN[4,5] and PAUL L. E. BODELIER[1]

[1]*Department of Microbial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Droevendaalsesteeg 10, 6708 PB Wageningen, The Netherlands,* [2]*Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch Straße 10, D-35043 Marburg, Germany,* [3]*SoilCares Research, Binnenhaven 5, 6709 PD Wageningen, The Netherlands,* [4]*Department of Terrestrial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Droevendaalsesteeg 10, 6708 PB Wageningen, The Netherlands,* [5]*Laboratory of Nematology, Wageningen University and Research Centre (WUR), PO Box 8123, 6700 ES Wageningen, The Netherlands*

Abstract

Intensification of agriculture to meet the global food, feed, and bioenergy demand entail increasing re-investment of carbon compounds (residues) into agro-systems to prevent decline of soil quality and fertility. However, agricultural intensification decreases soil methane uptake, reducing, and even causing the loss of the methane sink function. In contrast to wetland agricultural soils (rice paddies), the methanotrophic potential in well-aerated agricultural soils have received little attention, presumably due to the anticipated low or negligible methane uptake capacity in these soils. Consequently, a detailed study verifying or refuting this assumption is still lacking. Exemplifying a typical agricultural practice, we determined the impact of bio-based residue application on soil methane flux, and determined the methanotrophic potential, including a qualitative (diagnostic microarray) and quantitative (group-specific qPCR assays) analysis of the methanotrophic community after residue amendments over 2 months. Unexpectedly, after amendments with specific residues, we detected a significant transient stimulation of methane uptake confirmed by both the methane flux measurements and methane oxidation assay. This stimulation was apparently a result of induced cell-specific activity, rather than growth of the methanotroph population. Although transient, the heightened methane uptake offsets up to 16% of total gaseous CO_2 emitted during the incubation. The methanotrophic community, predominantly comprised of *Methylosinus* may facilitate methane oxidation in the agricultural soils. While agricultural soils are generally regarded as a net methane source or a relatively weak methane sink, our results show that methane oxidation rate can be stimulated, leading to higher soil methane uptake. Hence, even if agriculture exerts an adverse impact on soil methane uptake, implementing carefully designed management strategies (e.g. repeated application of specific residues) may compensate for the loss of the methane sink function following land-use change.

Keywords: atmospheric methane oxidation, bio-based residues, greenhouse gas, methane, methanotrophs, *pmoA*

Received 3 April 2015 and accepted 1 May 2015

Introduction

Meeting the food, feed, and bioenergy demand for the growing human population necessitates the conversion of native to arable land and/or intensification of agriculture, which entails increased residue input into agro-systems (Kai *et al.*, 2011; Werling *et al.*, 2014). The collection of carbon compounds (i.e. residues) from production land is typically re-invested as soil additives to improve soil quality and fertility. For practical and hygienic reasons, these residues are locally sourced. However, residue application in agro-systems may also cause higher greenhouse gas emissions (see review Thangarajan *et al.*, 2013). Recognized as a potent greenhouse gas, methane has a 34-fold higher global warming potential than carbon dioxide in a 20-year scale (IPCC, 2013). In particular, anomalies in atmospheric methane concentrations including the recent methane increase are a cause for global concern (Nisbet *et al.*, 2014). However, methane emissions can be attenuated by methanotrophic bacteria, acting as a bio-filter in wetland agricultural soils (rice paddies) or a methane sink in well-aerated soils.

In particular, atmospheric methane uptake in well-aerated soils is catalyzed by a specialized group of methane oxidizers ('high-affinity' methanotrophs) that are distinguished from known obligate 'low-affinity' methanotrophs. The putative 'high-affinity'

Correspondence: Adrian Ho, tel. +31 (0) 317473511, e-mail: a.ho@nioo.knaw.nl

methanotrophs associated with atmospheric methane oxidation could be affiliated to the *Gammaproteobacteria* (e.g. upland soil cluster γ: USC-γ, JR2, JR3, and TUSC) and *Alphaproteobacteria* (e.g. USCα, RA14, and JR1) (Knief *et al*., 2003; Horz *et al*., 2005; Kolb *et al*., 2005; Shrestha *et al*., 2012). As these methanotrophs have resisted cultivation so far and are only identified based on their *pmoA* gene sequences and associated lipid-labeling profiles (Bull *et al*., 2000; Knief *et al*., 2003; Singh *et al*., 2009), their physiological constituent and metabolic potential remain enigmatic. However, in a stable isotope labeling study, Pratscher *et al*. (2011) showed that putative methanotrophs belonging to the USCα clade can consume acetate as an alternative carbon source, testifying to the versatility in substrate utilization among some of the putative 'high-affinity' methanotrophs. The ability to oxidize methane at atmospheric or low methane (< 600 ppm_v) concentrations is also shared by some cultured alphaproteobacterial methanotrophs (e.g. *Methylocystis* spp.) where methane oxidation can be catalyzed by an isozyme of the conventional particulate methane monooxygenase, pMMO (pMMO2; Yimga *et al*., 2003; Baani & Liesack, 2008). Although not yet proven to oxidize atmospheric methane, the alphaproteobacteria methanotroph *Methylosinus* also has pMMO2 (Yimga *et al*., 2003). Similarly, *Methylocystis* possess versatility in substrate utilization (Belova *et al*., 2011; Im *et al*., 2011). It appears that methanotrophs oxidizing methane at trace levels may not be solely dependent on methane and may derive energy from alternative carbon sources. Apart from the cultivable alphaproteobacterial methanotrophs, the putative 'high-affinity' methane oxidizers are thought to form the vast active majority in well-aerated native soils (Knief *et al*., 2003; Kolb *et al*., 2005; Kolb, 2009; Pratscher *et al*., 2011).

While the methanotrophic potential in wetland agricultural soils (rice paddies) have been well documented (Ho *et al*., 2011a; Lee *et al*., 2014; Lüke *et al*., 2014; Leng *et al*., 2015), nonwetland (well-aerated) agricultural soils have received relatively little attention. This is presumably due to the anticipated low or negligible methane uptake capacity in these soils (Mosier & Delgado, 1997), and despite of their potential methane sink function. Here, we address the impact of agricultural practice exemplified by residue amendment on *in situ* soil methane flux and the methane oxidation potential in two agricultural soils (sandy loam and clay) representing typical agriculture lands in North-Western Europe and other temperate regions. For the organic amendments, we selected bio-based residues with a broad range of C:N ratio. Furthermore, we characterized the baseline methanotrophic composition using a diagnostic microarray analysis targeting the *pmoA* gene (a subunit of the genes encoding for the pMMO) and determined the shift in the abundance of specific methanotroph subgroups as well as the total bacterial population over time using group-specific quantitative PCR (qPCR) assays.

Materials and methods

Soils and soil additives (residues)

Agricultural soils were sampled from potato fields located in The Netherlands at Vredepeel (51°32′32″N, 05°50′54″E) and Lelystad (52°31′20″N, 05°34′57″E), representing sandy loam and clay soils, respectively. These soils typify agricultural lands in The Netherlands (Table 1) and in other temperate regions. The ploughed soil layer (0–10 cm) was collected after crop harvest (October 2013) from four 1 × 1 m plots at random and mixed to produce a composite sample. The soil was air-dried at ambient temperature in the greenhouse for approximately 2 months and sieved (< 2 mm) before use to minimize soil heterogeneity in the incubation setup. An aliquot of the soil was kept at 4 °C to determine the soil characteristics (Table 1). The residues (Table 2) comprised bio-based materials and included sewage sludge (Vallei Veluwe, The Netherlands), aquatic plant material of > 95% *Elodea Canadensis*, commercial compost (Recomede, The Netherlands), lignin-rich organic waste stream (designated 'wood material') after biological oxidation for energy generation, and compressed sugar beet leaves (Suiker Unie, The Netherlands). The residues were selected to represent a wide spectrum of C:N ratios ranging from 5.5 to 28.0 (Table 2) in the following order: sewage sludge < aquatic plant material < compost < wood material < compressed beet leaves. With the exception of the wood material, these residues were selected based on their ease of availability in large quantities for potential use as bio-based soil additives. These residues were oven-dried at 30 °C, crushed, and sieved (< 2 mm) before use. Both the soils and residues were dried and sieved prior to set up to ensure standardized initial incubation conditions by thorough mixing.

Experimental setup for in situ methane flux measurements

The soil (2.5 kg dry weight) and residues were mixed by hand in a pot giving a final working dimension of 22 × 10 cm (diameter × height), and deionized water was added to 65% of soil water retention capacity. The residue addition to the soil corresponded to a rate of 20-ton ha^{-1} typically used in agricultural practice (Diacono & Montemurro, 2010). Incubation was performed using six replicates for each treatment in a climate chamber at 15 °C (mean annual temperature in The Netherlands is 10 °C) in the dark for approximately 2 months (56 days). Water loss, measured by weight, was compensated for bi-weekly. Periodically, methane and carbon dioxide fluxes were measured under ambient air over an hour after placing the pot in a gas-tight

Table 1 Selected soil physico-chemical parameters

Soil texture (soil type)	Total C (μg C mg dw soil^{-1})	Total N (μg N mg dw soil^{-1})	C:N	Total soil nutrient contents (μg g dw soil^{-1})*			pH†	Water-holding capacity (%)	Density (g cm^{-3})	Organic matter content‡ (%)	Vegetation§
				NO_x	NH_4^+	PO_4^{-3}					
Sandy loam (Gley podzol)	22.20 ± 3.04	1.28 ± 0.21	17.30	3.94 ± 0.37	1.78 ± 0.02	0.87 ± 0.04	5.38 ± 0.02	26	1.67	4.74	Fallow (potato)
Clay (Calcareous clay)	26.25 ± 2.29	1.72 ± 0.17	15.27	5.39 ± 0.56	3.39 ± 0.10	0.25 ± 0.01	7.64 ± 0.02	36	1.25	4.79	Fallow (potato)

Measurements were performed in triplicate (mean ± SD).
*Sum of the soluble and adsorbed nutrient fractions in the soil. NO_x refers to the total of NO_2^- and NO_3^-.
†pH determined in 1 M KCl (1:5, vol:vol).
‡Organic matter content determined by loss on ignition (LOI %).
§During sampling, (in brackets, previous crop cultivated).

chamber (diameter × height: 24 × 40 cm) connected to an Innova 1412-5i Photoacoustic Infrared gas analyzer (lumaSense Technologies, Ballerup, Denmark). Sampling was automated using an Innova 1309 multiplexer gas sampling system equipped with a moisture trap (lumaSense Technologies). Gas flux from an un-amended pot placed in an empty chamber served as reference. The pots were left in the gas-tight chamber for 30 min prior to the first measurement to equilibrate soil – atmosphere gas exchange. The gas flux rate was determined by linear regression from at least four time intervals with a typical coefficient of determination, $R^2 > 0.8$ and $R^2 > 0.9$, respectively, for methane and carbon dioxide flux. After the gas measurement, the soil was sampled using a corer (diameter × height: 3 × 10–12 cm). The soil core was replaced by a plastic tube of a similar diameter to minimize disturbance to the soil. After sampling, sub-samples of the soil were stored in the −20 °C freezer and 4 °C fridge for later molecular and chemical analyses, respectively. An additional aliquot of the fresh soil (5 g) was sieved (< 2 mm) for incubation at near-atmospheric methane concentration (~40 ppm_v) to determine the potential methane oxidation rate.

Batch incubation setup for determination of apparent V_{max} and K_m, and potential methane oxidation rate

The apparent Michaelis–Menten constants for methane oxidation kinetic (V_{max} and K_m) were determined in triplicate in 260 ml opaque gas-tight bottles containing 5 g sieved (< 2 mm) fresh soil from the field. Methane was added to give final headspace concentrations of 20, 350, 2000, 4000, 12 000, 24 000, and 36 000 ppm_v (0.002–3.6 vol.% headspace CH_4). The apparent V_{max} and K_m values were derived from a plot fit using hyperbolic nonlinear regression using SIGMAPLOT version 12.5 (Systat Software Inc., San Jose, CA, USA). The bottles were incubated at 25 °C in the dark without shaking. To determine the potential methane oxidation rate in the un-amended and residue-amended soils over time, 5 g soil from the potted incubation was incubated without shaking in 120 ml bottles at 15°C in the dark with an initial headspace methane concentration of ~40 ppm_v.

Methane and soil nutrient determination

Headspace methane concentration in the bottled incubation was followed using an Ultra GC gas chromatograph (Interscience, Breda, The Netherlands) equipped with a Flame Ionization Detector (FID) and at Rt-Q-Bond (30 m, 0.32 mm, ID) capillary column. Helium was used as a carrier gas, and oven temperature was set at 80 °C.

Soil nutrient contents (NO_x, NH_4^+, and PO_4^{3-}) were determined in 1M KCl (1:5 dilution) extract using a SEAL QuAAtro SFA autoanalyzer (Beun- de Ronde B.V. Abcoude, The Netherlands). NO_x refers to the total of NO_2^- and NO_3^-. To determine the total carbon and nitrogen content, samples were oven-dried at 40 °C for 5 days before being ground and sieved (0.4 mm) for the Flash EA1112 CN analyzer (ThermoFisher Scientific, Breda, The Netherlands).

Table 2 Residue description, and total C and N contents

Residues	Total C (μg C mg dw sample^{-1})	Total N (μg N mg dw sample^{-1})	C:N	Description (Source/location)
Sewage sludge	322.03 ± 1.91	59.02 ± 0.40	5.46	Sampled from an anaerobic digester after sludge thickening (Vallei Veluwe, The Netherlands)
Aquatic plant material	368.24 ± 15.56	26.37 ± 0.13	13.97	Sampled from a ditch, comprise of > 95% Elodea Canadensis (Wageningen, The Netherlands)
Compost	139.71 ± 13.68	9.11 ± 0.32	15.33	Mature compost derived from organic materials e.g. for example, plant clippings and grass. (Recomede, The Netherlands)
Wood material	373.26 ± 29.45	21.86 ± 1.01	17.08	Low-grade wood material from a pilot bioreactor (Sustainable Winners and Department of Environmental Technology, Wageningen University, Netherlands)
Compressed beet leaves	383.96 ± 6.73	13.96 ± 0.41	27.50	Processing of sugar beet leaves mainly involves different stages of heating and de-watering, without chemical treatments (Suiker Unie, The Netherlands)

Measurements were performed in triplicate (mean ± SD).

Table 3 PCR primer and thermal profile used for qPCR assays

Primer set	Primer concentrations (forward/reverse)	PCR thermal profile*	Data acquisition	qPCR assay	References
A189f/Mb661r	875 nM/875 nM	94 °C, 10 s; 62 °C, 10 s; 72 °C, 25 s	87 °C, 8 s	MTOT	Kolb *et al.* (2003)
II223f/II646r	525 nM/525 nM	95 °C, 10 s; 60 °C, 10 s; 72 °C, 25 s	87 °C, 8 s	TYPEII	Kolb *et al.* (2003)
EUB338f/EUB518r	250 nM/250 nM	95 °C, 10 s; 53 °C, 10 s; 72 °C, 25 s	72 °C, 5 s	EUBAC	Fierer *et al.* (2005)

*Thermal profile showing temperature and time for denaturation, annealing, and elongation.

DNA extraction and qPCR assays

DNA was extracted in triplicate using the PowerSoil®DNA Isolation kit (MOBIO, Uden, The Netherlands) according to manufacturer's instruction per treatment, soil type, and time. We performed qPCR assays targeting methanotrophs (Kolb *et al.*, 2003): Alphaproteobacterial methanotrophs (TYPEII assay) and the total methanotrophic community (MTOT assay). In addition, we performed the EUBAC assay to enumerate the total bacterial 16s rRNA gene copies in the samples. Each assay was performed in duplicate for each DNA extract with primers, primer concentration, and PCR profiles as shown in Table 3. Briefly, each qPCR (total volume 20 μl) for the TYPEII and MTOT assays consisted of 10 μl 2× SensiFAST SYBR (BIOLINE, Alphen aan den Rijn, The Netherlands), 3.5 μl of forward and reverse primers each, 1 μl bovine serum albumin (5 mg ml^{-1}; Invitrogen, Breda, The Netherlands), and 2 μl diluted template DNA. The qPCR for the EUBAC assay (total volume 15 μl) consisted of 7.5 μl 2× SensiFAST SYBR (BIOLINE), 0.75 μl of forward and reverse primers each, 1.5 μl bovine serum albumin (5 mg ml^{-1}; Invitrogen), 1.5 μl DNase- and RNase-free water, and 3 μl diluted template DNA. Plasmid DNA from isolates was used for the calibration of the standard curve. In a pilot qPCR run, undiluted and diluted (10-, 50-, and 100-fold dilution) DNA was used to obtain the optimal target yield. Subsequently, template DNA was diluted 50-fold and 10-fold for the sandy loam and clay soil, respectively. The qPCR was performed with a Rotor-Gene Q real-time PCR cycler (Qiagen, Venlo, The Netherlands). Amplicon specificity was verified from the melt curve and further confirmed by gel electrophoresis showing a single band of the correct size in the pilot qPCR run.

Detection of mmoX gene

PCR amplification to detect the *Methylocella*-specific *mmoX* gene (encoding for the soluble methane monooxygenase) was performed using the mmoXLF/mmoXLR primer combination according to (Rahman *et al.*, 2011) with minor modifications (Ho *et al.*, 2013a).

Diagnostic microarray and statistical analyses

The diagnostic microarray analysis targeting the *pmoA* gene was performed as previously described (Bodrossy *et al.*, 2003) with minor modifications (Ho *et al.*, 2011b) using the primer combinations A189f/T7_A682r and A189f/T7_A650r. The signal intensity of the microarray analysis was normalized against the mean total array intensity, and then, against a reference value experimentally determined for positive hybridization signals (Bodrossy *et al.*, 2003). A subset of probes representing the overall diversity of the methanotrophs covered by the microarray was used for the statistical analysis of the initial methanotroph community

Fig. 1 Methane flux in un-amended sandy loam (a) and clay (b) soils, and after amendments with sewage sludge, aquatic plant material, compost, wood material, and compressed beet leaves (mean ± SD; $n = 6$). Methane flux measurements were performed under ambient gas concentrations. In (c), the mean total methane emitted or consumed during incubation (56 days) was determined from the area below the curve. Note the different scale in the *y*-axis.

(Lüke *et al.*, 2014). The microarray analysis was visualized as a heatmap and constrained ordination analysis was produced in R software, version 2.10.0 (Development Core Team R, 2012) as implemented in the packages gplots (Warnes *et al.*, 2013) and vegan (Oksanen *et al.*, 2015), respectively. Evaluation for significance between treatments was performed using *t*-test in SIGMAPLOT version 12.5 (Systat Software Inc.).

Results

The abiotic environment

The sandy loam and clay soils had comparable total carbon and nitrogen contents, with C:N ratios of 17.3 and 15.3, respectively (Table 1). The sandy loam soil was slightly acidic (pH 5.4), whereas the clay soil was circum neutral (pH 7.6). The pH shifted within 1.1 units during incubation, with a pH range of 4.9–6.0 and 7.3–7.6 in the sandy loam and clay soils, respectively. With the exception of the sewage sludge- and aquatic plant material-amended incubations, NO_x and NH_4^+ concentrations remained relatively stable (Fig. S1a,b,c, d). The changes in NO_x and NH_4^+ concentrations which showed a decrease in NH_4^+, while NO_x increased suggest nitrification, particularly after amendment with sewage sludge, a nitrogen-rich substrate (~60 μg total N mg dw^{-1}) for the soil microorganisms. Sewage sludge addition also increased total phosphate in the sandy loam soil until day 20, but phosphate concentration was relatively constant in other residue-amended soils (Fig. S1e,f). Phosphate derived from the sewage sludge contributed substantially to both soils (18–20 μg g dw $soil^{-1}$; Fig. S1e,f).

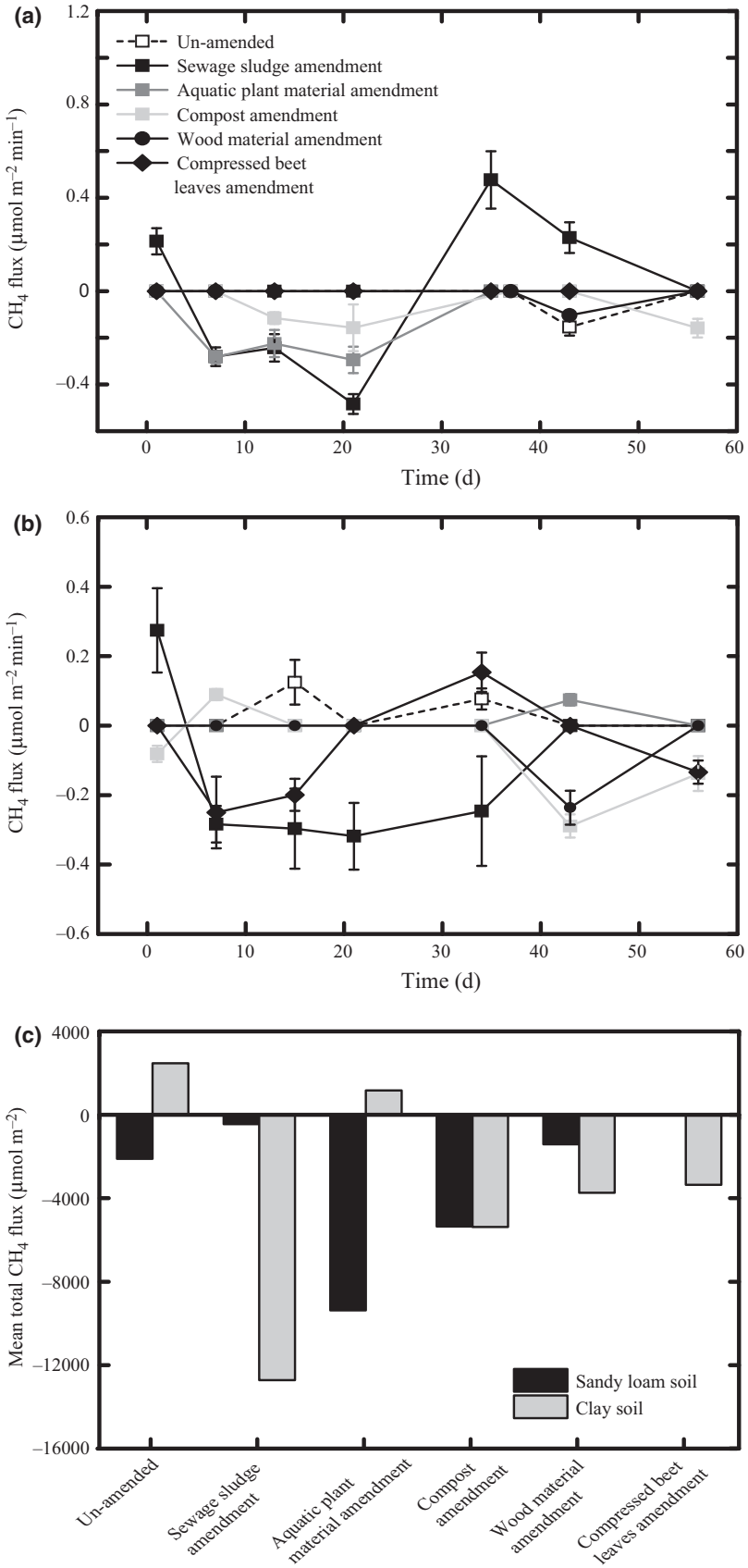
Methane flux measurements

Both soils acted as methane sinks and sources, depending on residue amendment and incubation time. Integrated over the total incubation (56 days), the un-amended sandy loam and clay soil showed an overall negative and positive methane flux, respectively. However, these values fluctuated with time, and generally, no appreciable methane fluxes were detected at individual time points (Fig. 1). Upon amendment with specific residues, methane uptake increased strongly in both soils (Fig. 1a,b). The apparent stimulation was transient and remained for approximately 35 and 45 days in the sandy loam and clay soils, respectively. Not all the residues induced higher methane uptake. The stimulatory effect was observed after amendment with sewage sludge, aquatic plant material, and compost in the sandy loam soil, and sewage sludge, compost, and compressed beet leaves in the clay soil (Fig. 1a,b); the sewage sludge and compost being the common residues to induce higher methane uptake in both soils. Sewage sludge amendment in the sandy loam soil would have appreciably increased total methane uptake if not offset by methane production after 21 days. Other residues do not appear to affect or have a consistent or appreciable effect on the net methane flux. Hence, further batch incubations were performed using the un-amended soils, as well as soils amended with sewage sludge, aquatic plant material, and compost to determine the potential methane oxidation rate.

Potential methane oxidation rate in un-amended and residue-amended soils

The agricultural soils exhibited the potential for methane oxidation over a broad concentration range from near atmospheric (20 ppm_v) up to high (> 10 000 ppm_v) concentrations (Fig. S2). Methane depletion curve showed a biphasic pattern in incubations under initial high methane concentrations. Hence, the potential methane oxidation rate was derived from the initial linear decrease over time (3–4 days), reflecting on the *in situ* active part of the population (Steenbergh *et al.*, 2010). The apparent substrate half saturation constants (K_m) were 16 000 and 4000 ppm_v, respectively, for the sandy loam and clay soil. These K_m values (> 175 μM) are indicative of methane oxidization at high methane concentrations, in contrast to soils exhibiting atmospheric methane uptake typically in a lower K_m (nM range; Singh *et al.*, 2010).

The net methane flux is a balance of methane production and oxidation. To determine whether the negative methane flux (Fig. 1a,b) was a result of higher methane oxidation rate following residue addition, the potential methane oxidation rate was monitored over time in the residue-amended soils and compared with the un-amended soil (Fig. 2). Methane oxidation rate increased significantly (*t*-test; $P < 0.05$) after residue addition and was more pronounced in the sandy loam soil (Fig. 2a). The higher potential for methane oxidation was sustained up to 21 days after sewage sludge amendment


(a)
Un-amended
Sewage sludge amendment
Aquatic plant material amendment
Compost amendment
Wood material amendment
Compressed beet leaves amendment
CH4 flux (μmol m−2 min−1)
Time (d)
(b)
CH4 flux (μmol m−2 min−1)
Time (d)
(c)
Mean total CH4 flux (μmol m−2)
Sandy loam soil
Clay soil
Un-amended
Sewage sludge amendment
Aquatic plant material amendment
Compost amendment
Wood material amendment
Compressed beet leaves amendment

in both soils and diminished at the end of incubation (56 days). In the clay soil, amendment with aquatic plant material had relatively little effect on the methane oxidation rate, whereas this residue significantly stimulated methane oxidation rate in the sandy loam soil (Fig. 2).

The pmoA gene diversity

The microarray analysis was performed on both soils prior to the addition of residues using two primer combinations to capture the methanotroph diversity (Fig. 3). The microarray has an extensive coverage of isolated methanotrophs as well as those identified only by their *pmoA* sequences, including the *pmoA* clusters associated with atmospheric methane oxidization (full probe coverage; Ho *et al*., 2013b). The primer combination A189f/T7_A682r is not methanotroph specific and detected sequences affiliated to *amoA* belonging to ammonium oxidizers (potentially, probe gp23.454) due to the homology of the methane and ammonium monooxygenases (Fig. 3). Other positive hybridization signals include those for the alphaproteobacterial methanotrophs (*Methylosinus*; probe Msi294) as well as the *pmoA2* belonging to *Methylosinus trichosporium* (probe NMsiT.271), gammaproteobacterial methanotrophs (probes LP20.644, Mc396, and LW21.391), and *pmoA* related to the tropical upland soil clusters (probes TUSC409 and TUSC502). Besides probe LW21.391 which gave a strong hybridization signal only in the sandy loam soil, the other probes showed a relatively weak hybridization signal. The primer pair A189f/T7_A650r specifically targets methanotrophs and hybridized exclusively to probes indicative for *Methylosinus* and its related *pmoA2* (probes Msi294 and NMsiT.271) in both soils. The microarray analysis revealed a low or undetectable hybridization signal for probes specific for the gammaproteobacterial methanotrophs using the methanotroph-specific primer combination. This indicated relatively low methanotroph diversity in these agricultural soils (Lee *et al*., 2014; Lüke *et al*., 2014). Probes indicative for *Methylosinus* and *pmoA2* were detected in both soils, and this was consistent across both primer combinations. Bias caused by the different primer sets was evident, but not between soil types (Fig. S3). Moreover, we did not retrieve amplicons of the correct size in the PCR targeting the soluble methane monooxygenase (i.e. *Methylocella*-affiliated *mmoX* gene).

16S rRNA and pmoA gene abundance

The methanotroph-specific microarray analysis revealed a high relative abundance of alphaproteobacterial methanotrophs in both soils. Subsequently, we performed qPCR assays targeting the *pmoA* gene of the alphaproteobacterial and total methanotrophs. In addition, a qPCR assay targeting the universal 16s rRNA gene was performed to enumerate the total bacteria in the soil. Considering that the methane flux and methane oxidation rate were more responsive to and differentially affected by specific residue amendments (Figs 1 and 2), the qPCR assays were performed for the sewage sludge-, aquatic plant material-, and compost-amended soils over time, as well as for the un-amended incubation.

Both soils harbored comparable *pmoA* and 16S rRNA gene abundances, with the 16S rRNA gene copies being four- to fivefold magnitude higher than the *pmoA* gene abundance (Fig. 4a,b). The *pmoA* gene copies specific for the alphaproteobacterial methanotrophs (TYPEII assay) in the residue-amended and un-amended incubations were of similar magnitude, indicating that the soils, rather than the residues, harbored the vast majority of alphaproteobacterial methanotrophs, supporting the microarray analysis. As anticipated, the total *pmoA* copies (MTOT assay) were either comparable to or a magnitude higher than in the residue-amended incubations. The discrepancy in the initial total *pmoA* gene copies in the un-amended and residue-amended soils, however, did not persist; both total and alphaproteobacterial methanotroph *pmoA* copies remained relatively constant over time (Fig. 4). Applying a correspondence analysis on the qPCR data (TYPEII, MTOT, and EUBAC assays) using environmental variables as constraints (i.e., methane flux, methane oxidation rate, carbon dioxide flux, NO_x, NH_4^+, PO_4^{3-}, incubation time, and soil type) revealed only a weak, but statistically significant correlation for the methane and carbon dioxide fluxes, incubation time, and NH_4^+ ($P = 0.025$). Together they explained 32.4% of the total variance (Fig. S4). Although not appreciable, a trend showing an increase of the TYPEII assay at the start of the incubation is discernible. With the exception of sewage sludge amendment in the sandy loam soil, this trend was consistent in the other residue-amended incubations. In the MTOT assay, total *pmoA* copy numbers decreased with time (< 14 days) in the residue-amended incubations, but remained relatively constant, and were comparable to the TYPEII assay in the un-amended incubation.

Discussion

The abandonment of agriculture and subsequent conversion to nonagricultural lands (e.g., afforestation) has resulted in an increase in soil atmospheric methane uptake or methane oxidation rates (Priemé *et al*., 1997;

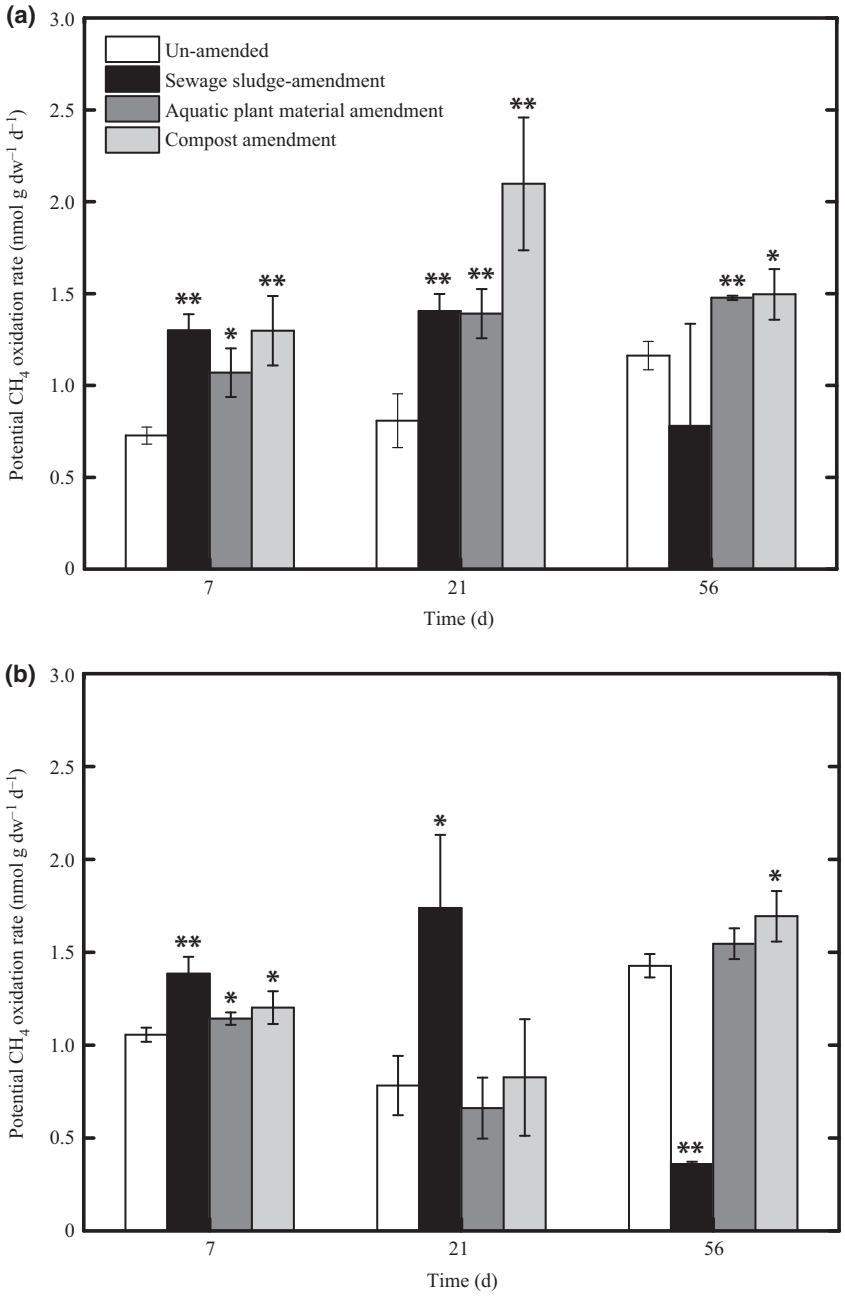


Fig. 2 Potential methane oxidation rates in un-amended sandy loam (a) and clay (b) soils, and after amendments with sewage sludge, aquatic plant material, and compost. Level of significance is indicated by asterisk (*t*-test; $*P < 0.05$, $**P < 0.005$) as determined by comparing the rates in the residue-amended incubations to the reference (un-amended soils) per time.

Levine *et al*., 2011; Nazaries *et al*., 2011; Hiltbrunner *et al*., 2012). Indeed, well-aerated native soils are recognized methane sinks, and have, on average, a higher atmospheric methane uptake capacity than agricultural soils (Keller *et al*., 1990; see review Tate, 2015). In contrast, our results showed that the application of bio-based residues in agricultural soils significantly stimulated methane oxidation rate, markedly increasing soil methane uptake to rates higher than in well-aerated native soils.

Response of methanotrophic activity to residue amendments

The agricultural soils showed methane oxidation potential over a wide range of methane concentrations

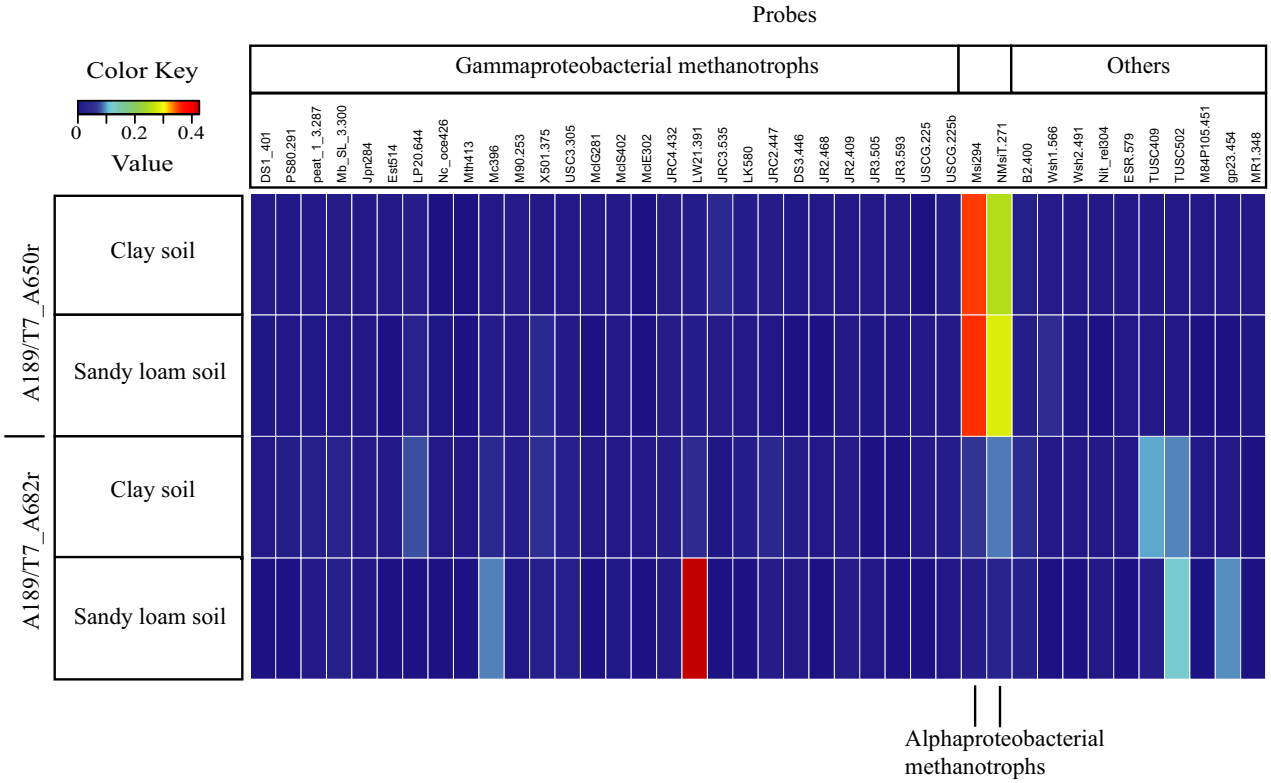


Fig. 3 The diagnostic microarray analysis visualized as a heatmap showing the diversity of the methanotrophic composition in the soils determined from two primer combinations. The A189f/T7_A650r primer pair is methanotroph-specific, whereas the A189f/T7_A682r primer pair also covers the ammonium-oxidizing bacteria, besides the methanotrophs. The microarray analysis was derived from independent triplicate measurements for each sample and given as average. The analysis for each replicate is given in Fig. S4. The hybridization signal is represented by a color code, with blue and red showing the lowest and highest relative abundance, respectively. The full coverage of the probes and designated specificity are given elsewhere (Ho *et al.*, 2013b).

(20 – > 10 000 ppmv), indicating a methanotrophic community capable of methane oxidation at near-atmospheric and high methane concentrations in these soils. Similarly, other well-aerated native (e.g. forest soils) and agricultural soils have shown the capacity to oxidize methane over a wide concentration range (Menyailo *et al.*, 2008; Shrestha *et al.*, 2012; Ho *et al.*, 2013a), which may reflect seasonal fluctuations in methane concentrations (Horz *et al.*, 2002), and indicate the occurrence of methanogenesis. Hence, methanotrophs in our agricultural soils may benefit after a rain event when methane production is stimulated. These soils may act as a bio-filter to mitigate methane emission and/or serve as an atmospheric methane sink, depending on the prevailing environmental conditions.

Interestingly, amendment with specific residues significantly increased methane oxidation rate in both the agricultural soils, but the apparent stimulation was transient. Reviewing the literature, the general consensus is that well-aerated native (undisturbed) soils, on average, possess higher methane uptake capacity than agricultural soils within study sites (Table 4). The decrease in methane uptake in agricultural soils has been attributed to the destruction of the soil physical structure and stratified zones (e.g. ploughing, soil compaction), and disturbance induced by other agricultural practices (e.g. fertilization) following land-use change (Bender & Conrad, 1992; Boeckx *et al.*, 1997; Hiltbrunner *et al.*, 2012). Unexpectedly, after amendment with specific residues, the agricultural soils in our study became a stronger net methane sink independent of soil physical factors (Fig. 1a,b), exhibiting methane uptake values up to threefolds higher (< 21 days; up to 29 μmol m^{-2} h^{-1}) than in well-aerated native soils (Table 4). In particular, the clay soil turned from a net methane source to sink (Fig. 1c). Our agricultural soils showed potential methane oxidation rates comparable to native soils from wide geographic regions after amendment with specific residues. By contrast, potential methane oxidation rates in the un-amended incubations can be an order of magnitude lower than in well-aerated native soils (Table 4). Depending on the

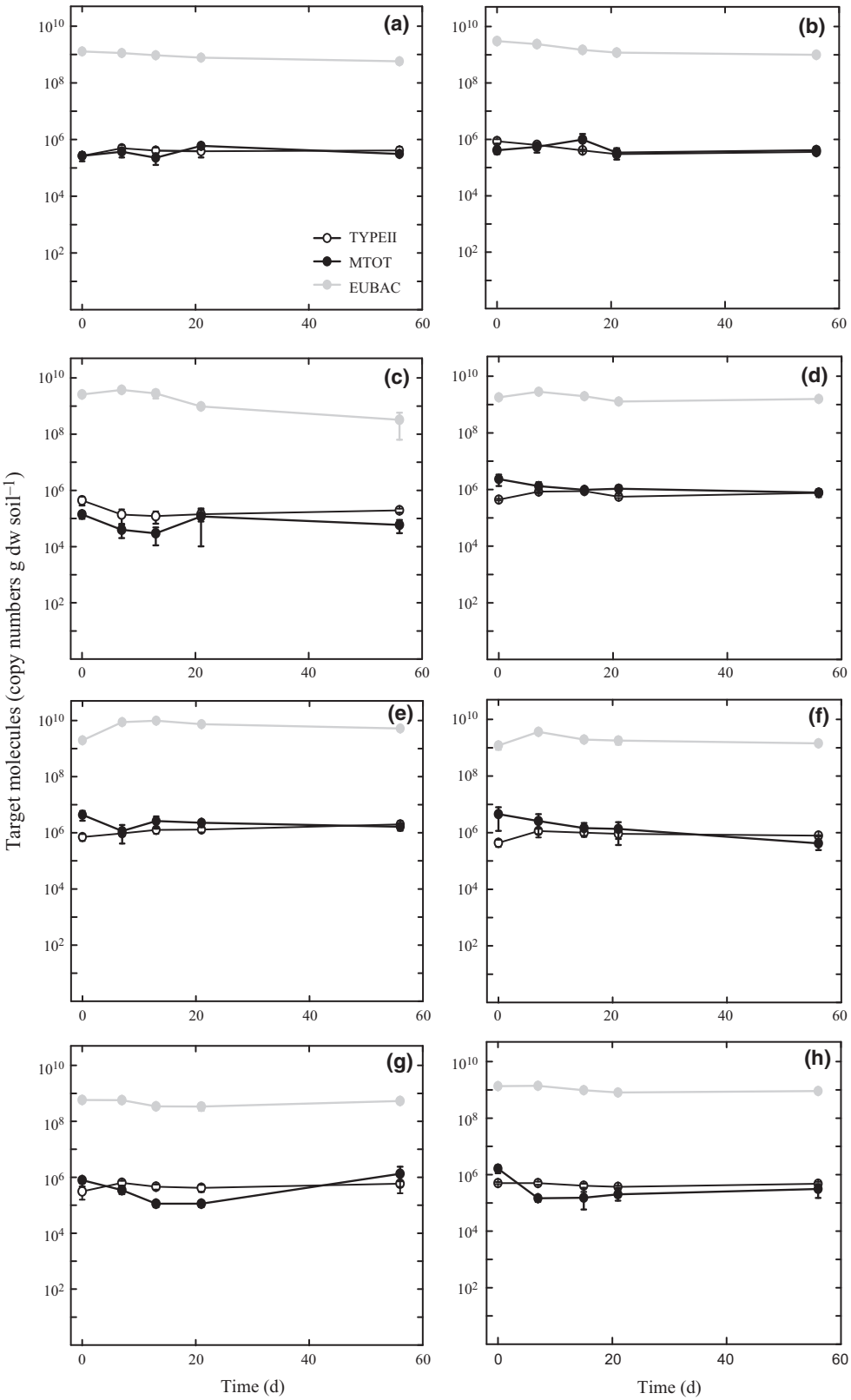


Fig. 4 Changes in the *pmoA* (TYPEII and MTOT assays) and 16s rRNA (EUBAC assay) gene abundances (mean ± SD; $n = 6$) in the un-amended soils (a,b), and sewage sludge (c,d)-, aquatic plant material(e,f)-, and compost (g,h)-amended soils. The left and right panels are sandy loam (a,c,e,g) and clay (b,d,f,h) soils, respectively.

predominant process – methanogenesis or methane oxidation – the agricultural soils can act as a net methane source or sink, resulting in no apparent flux as has been observed in the un-amended soils. This explains the detection of a potential methane oxidation rate despite no apparent methane flux being observed from the un-amended soil (Fig. 1a,b). Previously, we detected methane production under anoxic condition in the clay soil, and the methane production rate significantly increased after manure fertilization, showing that the methanogenic activity can be substantial after residue application in this soil (Ho *et al.*, 2015). Therefore, considering the results from the methane flux measurement and methane oxidation assay together strongly suggest a stimulation of methane oxidation rates leading to a stronger net negative methane flux, whereas no detectable net methane flux indicates that methane produced was completely consumed.

Residue as a source of methanotrophs and nutrients

Residue addition increases bio-available nutrients and/or introduces methanotrophs indigenous to the residue into the soil, thereby stimulating methanotrophic activity. Notably, fresh sewage sludge and aquatic plant material are known to harbor a high abundance of methanotrophs (10^7–10^8 *pmoA* copies g volatile suspended solids^{-1}: Ho *et al.*, 2013b; 10^5–10^7 *pmoA* copies g dry weight plant material^{-1}: Yoshida *et al.*, 2014) and is reflected in the qPCR analysis which showed comparable or higher total *pmoA* gene copies in the residue-amended soils (Fig. 4). Besides, residue addition effectively contributed to the higher nutrient availability in the agricultural soils (Fig. S1), which in turn, stimulated the methanotrophic activity soon after amendment (Figs 1 and 2). This was consistent with the CO_2 emission rate which was most responsive immediately after residue addition (sewage sludge, aquatic plant material, and compressed beet leaves), and the higher respiration rate was sustained for < 21 days (Fig. S5). Although we do not anticipate methanotrophic activity in the residues which were air-dried prior to mixing in the soil, we cannot entirely exclude the contribution of the residue-derived methanotrophs which are likely to be present as a reservoir of resting cells to the total methane uptake.

The apparent residue-induced methane uptake indicates that the methanotrophs, particularly the soil-borne population, were nutrient limited. The effects of nutrient availability (e.g. nitrogen) on the methanotrophic activity vary, depending on the methanotrophic composition (i.e. 'low-' and 'high-affinity' methanotrophs), and environmental conditions (see review Semrau *et al.*, 2010; Ho *et al.*, 2013c). Among the nutrients measured (NO_x, NH_4^+, and PO_4^{3-}), the methanotroph and bacteria abundances were found to be significantly affected only by the NH_4^+ concentration, which coincides with the carbon dioxide flux (Figs S4 and S5a,b). Depending on the type of residues (Table 2), ~23–148 g total N was added into the soils. NH_4^+ amendment exerts a differential response in methanotrophs. NH_4^+ has been shown to (in)directly inhibit atmospheric methane uptake in well-aerated native soils presumably from the realm of 'high-affinity' methanotrophs (Schnell & King, 1994; Mosier & Delgado, 1997; Bodelier & Laanbroek, 2004). The reverse is true, however, in a wetland rice paddy inhabited by predominantly 'low-affinity' methanotrophs, where NH_4^+ stimulated methane uptake and selected for specific methanotroph subgroups (Bodelier *et al.*, 2000; Noll *et al.*, 2008). It is thought that methanotrophs in agricultural soils are nitrogen limited due to strong competition for nutrients by plants, but upon relief of nitrogen limitation, the methanotrophs responded immediately to the available nitrogen, suggesting a yet unknown mechanism regulating nitrogen metabolism (Bodelier *et al.*, 2000; Bodelier, 2011). Besides nitrogen, methanotroph abundance may be restricted by PO_4^{3-}, as observed in an Arctic soil (Gray *et al.*, 2014). However, the effects of PO_4^{3-} on the methanotrophic activity and composition are less well known (Veraart *et al.*, 2015).

The pmoA gene diversity and abundance in nonwetland agricultural soils

In contrast to wetland agricultural soils (rice paddy), the well-aerated agricultural soils harbored a less diverse methanotrophic community (Fig. 3; Lee *et al.*, 2014; Lüke *et al.*, 2014), comprising almost exclusively of *Methylosinus*, as revealed in the microarray analysis using methanotroph-specific primer combination. With the exception of probe LW21.391, gammaproteobacterial methanotrophs were not detected or detected in low relative abundance in both soils. The microarray analysis was confirmed by qPCR assays targeting the gammaproteobacterial *pmoA* (MBAC and MCOC assays; Kolb *et al.*, 2003) in the starting material, showing the *pmoA* copies to be below the detection limit or unspecific amplification (data not shown). Probe LW21.391 is indicative of putative methanotrophs so far not known to oxidize methane at trace levels. Although relatively abundant in the sandy loam soil, they are unlikely to play a significant role in our incubations under near-atmospheric methane levels. Moreover, probes indicative of upland soil clusters (Horz *et al.*, 2002; Knief *et al.*, 2003) and other known communities associated with atmospheric methane oxidation thought to be relevant in many upland soils, did not show any

Table 4 Methane flux and potential methane oxidation rate of well-aerated native and agricultural soils from widespread geographic origins

Land use (description)	Geographic origin	Soil type (soil texture)	pH	C:N	Potential methane oxidation rate				Methane flux*		References
					Soil depth (cm)	Incubation temperature (°C)	Initial CH_4 (ppmv)	CH_4 oxidation rate (nmol g dw^{-1} h^{-1})	Sampling period	CH_4 flux (μmol m^{-2} h^{-1})	
Native soils											
Shrubland	Turangi, New Zealand	n.a	5.5	20–21	n.a	n.a	n.a	n.a	February	−5.00 to −6.25	Nazaries *et al.* (2011)
Forest	Puruki, New Zealand	n.a	5.0	17	n.a	n.a	n.a	n.a	January	~−9.38	Nazaries *et al.* (2011)
Forest	Hesse, Germany	Cambisoil (loam)	4.3	30	0–4	25 °C	1.9	~0.31	November–April	−2.08 to −8.66	Kolb *et al.* (2005)
Forest	Lower Saxony, Germany	Gleyic luvisol (silty clay)	7.7	18	0–4	25 °C	1.9	~0.05	June–November	−2.50 to −3.91	Kolb *et al.* (2005)
Forest	Fribourg, Switzerland	Cambisols (loam)	3.9–4.9	n.a	0–20	20 °C	Ambient air	0.01–0.07	July–October	−1 to −5.5	Hiltbrunner *et al.* (2012)
Forest	Belgium	n.a	3–4.5	n.a	0−10	25 °C	10	n.a	October	~−1.00	Boeckx *et al.* (1997)
Tropical forest	Barro Colorado Island, Panama	Oxisol (clay)	n.a	n.a	0–10	25–27 °C	Ambient air	~0.06	June and September	−2.03	Keller *et al.* (1990)
Tropical forest	Barro Colorado Island, Panama	Alfisol (clay)	n.a	n.a	0–10	25–27 °C	Ambient air	~0.05	May and September	−0.91	Keller *et al.* (1990)
Woodlands	Lubumbashi, Democratic Republic of Congo	Ferralsols (clay)	5.0 –5.4	n.a	0–10	28 °C	40	0.02–0.03	n.a	n.a	Ho *et al.* (2013a)
Deciduous forests	Michigan, United states of America	Typic hapludalfs (loam)	n.a	n.a	n.a	n.a	n.a	n.a	March and December	~−2.78	Levine *et al.* (2011)
Grassland	Krasnoyarsk, Siberia, Russia	Grayzem (n.a)	6.1	13.8	0–10	22–24 °C	30 200 1000	~0.63 ~0.83 1–4.17	May–September	−3.91 to −10.42	Menyailo *et al.* (2008)
Agricultural soils											
Pasture	Rangipo, New Zealand	Orthic Pumice (sandy loam)	5.2–5.6	n.a	n.a	n.a	n.a	n.a	January	−2.42 to −2.72	Singh *et al.* (2009)
Pasture	Massey, New Zealand	Typic orthic brown (silt loam)	5.2–5.6	n.a	n.a	n.a	n.a	n.a	January	−0.08 to −1.13	Singh *et al.* (2009)
Pasture	Las Pavas, Panama	Oxisol (clay)	n.a	n.a	0–10	25–27 °C	Ambient air	~0.02	July and August	−0.36	Keller *et al.* (1990)

Table 4 (continued)

Land use (description)	Geographic origin	Soil type (soil texture)	pH	C:N	Potential methane oxidation rate				Methane flux*		References
					Soil depth (cm)	Incubation temperature (°C)	Initial CH_4 (ppmv)	CH_4 oxidation rate (nmol g dw^{-1} h^{-1})	Sampling period	CH_4 flux (μmol m^{-2} h^{-1})	
Upland rice and maize	Las Pavas, Panama	Oxisol (clay)	n.a	n.a	0–10	25–27 °C	Ambient air	~0.02	July and August	−0.57	Keller *et al.* (1990)
Conventional row-crop	Michigan, United states of America	Typic hapludalfs (loam)	n.a	n.a	n.a	n.a	n.a	n.a	March and December	~−0.35	Levine *et al.* (2011)
Cropland	Vestskoven, Denmark	Mollic Hapludalfs (sandy loam)	~6.1	10.0	0–15	20–22 °C	Ambient air	0.002–0.004	May	n.a	Bárcena *et al.* (2014)
Grassland (non-grazed, managed land)	Giessen, Germany	Stagno-fluvic gleysol (sandy loam)	5.8 – 6.5	n.a	0–10	25 °C	n.a	n.a	February – May	−1.3 to −2.9	Horz *et al.* (2002)
							10	†0.5–2.8 × 10^3	January – December	0 to −4.4	Shrestha *et al.* (2012)
							100	†3.9–25.5 × 10^3			
							1000	†2.65–210.3 × 10^3			
							10000	†22.3–323.5 × 10^3			
Arable land	Belgium	n.a	6–7.6	n.a	0–10	25 °C	10	n.a	October	−0.3 to −0.9	Boeckx *et al.* (1997)
Cropland (Un-amended)	Vredepeel, The Netherlands	Gley podzol (sandy loam)	5.4	17.3	0–10	15 °C	20	0.07	September	0 to +9.3	This study
Cropland (residue amendment)	Vredepeel, The Netherlands	Gley podzol (sandy loam)	5.4	17.3	0–10	15 °C	30–40	up to 0.09	September	−29.0 to +28.6	This study
Cropland (Un-amended)	Lelystad, The Netherlands	Calcareous clay (clay)	7.6	15.3	0–10	15 °C	20	0.05	September	+4.6 to +7.5	This study
Cropland (residue amendment)	Lelystad, The Netherlands	Calcareous clay (clay)	7.6	15.3	0–10	15 °C	30–40	up to 0.07	September	−19.1 to +16.5	This study

n.a; not available.

*Given as mean or range of methane flux rate, based on both field and laboratory flux measurements.

†Initial rates of methane oxidation potential (methane uptake from days 0 to 4) based on batch slurry incubations normalized to weight of soil.

hybridization signal, or exhibited only a relatively weak and inconsistent hybridization signal (probes TUSC409 and TUSC502). Despite their low relative abundance, we cannot exclude the potential role of the putative 'high-affinity' methanotrophs represented by the TUSC409 and TUSC502 probes to total methane uptake in these agricultural soils. The methanotrophic community composition in both soils, however, were similar despite being from different soil types and showed considerable overlap in their composition as revealed by a cluster analysis performed on standardized microarray data (Fig. S3). This suggests that the community composition was independent of the soil type. Recurring agricultural practices may be a stronger driving force shaping the methanotrophic community in these soils, as has been suggested for a rice paddy soil (Ho *et al*., 2011a).

Given the high apparent K_m indicates the ability to oxidize methane at near-atmospheric concentrations (20 ppm_v), but also suggests a broad methane utilization spectrum restricted to specific methanotroph subgroups. Beside the yet unculturable 'high-affinity' methane oxidizers, methanotrophs belonging to the *Alphaproteobacteria* notably some *Methylocystis* and *Methylosinus* species are known to harbor an isoenzyme of the conventional pMMO–pMMO2 (Yimga *et al*., 2003). While no growth has been detected at atmospheric methane levels, nonetheless, pMMO2 allows methane oxidation and growth at < 100 ppm_v methane concentration (Baani & Liesack, 2008). Hence, the detection of predominantly alphaproteobacterial methanotroph was consistent with the methane oxidation characteristics in the agricultural soils. Moreover, facultative methanotrophs are so far confined to the *Alphaproteobacteria*, with some *Methylocystis* species known to oxidize and grow on acetate and ethanol besides methane (Belova *et al*., 2011; Im *et al*., 2011; Leng *et al*., 2015). Hence, amendment with readily degradable residues (e.g. sewage sludge) may also provide extraneous assimilable carbon sources for the facultative methanotrophs, resulting in a transient stimulation of activity. Based on their ecological characteristics, *Methylosinus* have been suggested to be stress tolerant (Ho *et al*., 2013c), which may have contributed to their resilience and persistence in agriculturally impacted landscapes. Taken together, *Methylosinus* species may be indicative for our agricultural soils, but their occurrence cannot be taken for granted in other nonwetland agro-systems without confirmation.

In agricultural wetland soils (rice paddy), shifts in the potential for methane oxidation have been associated not only with the changes in the methanotrophic community, but also to the abundance of specific methanotroph populations (Ho *et al*., 2011a). Considering that alphaproteobacterial methanotrophs were consistently detected in both soils, qPCR assay specifically targeting the *pmoA* gene of this subgroup (TYPEII assay) was performed, along with an assay targeting all methanotrophs (MTOT assay) and the total bacteria population (EUBAC assay) in the soil. Given the low methane concentration at trace level (2–6 ppm_v) during the flux measurements and the slow growth rate of methanotrophs under atmospheric methane levels (Menyailo *et al*., 2008; Levine *et al*., 2011), we do not anticipate appreciable changes in the *pmoA* gene copies. Nevertheless, the qPCR analyses could be correlated with the methane flux (Fig. S4). An initial higher number of *pmoA* gene was detected, but the total *pmoA* gene abundance generally decreased (< 14 days) and remained relatively constant over time (Fig. 4), indicative of a stable methanotrophic community. While *pmoA* gene copies showed no appreciable changes with time regardless of the methanotroph origin, the increase in methane oxidation rate suggests a transient increase in cell-specific activity. This increase may also have resulted from activation of the dormant microbial population caused by nutrient availability rather than by growth.

Agricultural impact on the methane sink function in agricultural soils

Atmospheric methane oxidization is sensitive to disturbances (Mosier & Delgado, 1997; Roslev *et al*., 1997; Menyailo *et al*., 2008; Levine *et al*., 2011; Tate, 2015). Extrapolation of chronosequence studies indicates that around a century is needed for soil atmospheric methane uptake to recover after the conversion of agriculture land to grassland, or following afforestation (Menyailo *et al*., 2008; Levine *et al*., 2011). Atmospheric methane uptake in well-aerated native soils is thought to be facilitated by the elusive 'high-affinity' methane oxidizers. In contrast, our soils harbored a low methanotroph diversity mainly characterized by known *Methylosinus*, also thought to be able to oxidize methane at trace concentrations. These methanotrophs were seemingly not susceptible to agricultural practices, as indicated by the unexpected methane uptake capacity and stimulation by the addition of specific residues. However, how relevant is the stimulation in soil methane uptake for the overall greenhouse gas C budget (carbon dioxide and methane)? Assuming that one unit of methane is equivalent to 34 units of carbon dioxide in a 20-year scale (IPCC, 2013), and normalizing to the proportion of C mass in carbon dioxide (0.27), methane uptake would offset 0–16% of net emitted carbon dioxide in soils showing an overall methane uptake. Consistent in both soils, amendment with compost showed

the highest offset at ~16%, while values for other amendments ranged from 0–3.5% to 1–10%, respectively, in the sandy loam and clay soils. The compost could thus be applied in both agricultural soils to potentially reduce the impact of greenhouse gas (carbon dioxide and methane) emission. Nevertheless, considering the potential for nitrification activity, future studies could consider the impact of compost application on N_2O emission which also contributes to the greenhouse gas effect. Moreover, the present findings could be confirmed and extrapolated to a broader scale supported by field-based studies.

Taken together, while agricultural soils have generally been regarded as a net methane source or a relatively weak methane sink (Table 4), our results show that in contrast to this assumption, methane oxidation rate can be stimulated, leading to higher methane uptake in these soils that can exceed values reported for well-aerated native soils from widespread geographic regions. Hence, even if agriculture exerts an adverse impact on soil methane uptake, implementing carefully designed management strategies (e.g. repeated application of compost) may compensate for the loss of the methane sink function following land-use change.

Acknowledgements

We are grateful to Iris Chardon, Alaa El-Hawwary, and Erik van der Brink for excellent technical assistance. We extend our gratitude to Sascha Krause (University of Washington, USA) for assistance during sampling and fruitful discussion. We thank Willem van Marten (Recomede, The Netherlands) for providing us with the compost and Rob Verheijn (Vallei Veluwe, The Netherlands) for providing us with the sewage sludge. We also kindly acknowledge Michiel Verhofstad's (NIOO-KNAW, The Netherlands) expertise in identifying freshwater aquatic plants. AH is financially supported by the BE-Basic grant F03.001 (SURE/SUPPORT). This publication is publication no. 5859 of The Netherlands Institute of Ecology.

Conflict of interest

The authors declare no conflict of interest.

References

Baani M, Liesack W (2008) Two isozymes of particulate methane monooxygenase with different methane oxidation kinetics are found in *Methylocystis* sp. strain SC2. *Proceedings of the National Academy Sciences of the United States of America*, **105**, 10203–10208.

Bárcena TG, D'Imperio L, Gundersen P, Vesterdal L, Priemé A, Christiansen JR (2014) Conversion of cropland to forest increases soil CH_4 oxidation and abundance of CH_4 oxidizing bacteria with stand age. *Applied Soil Ecology*, **79**, 49–58.

Belova SE, Baani M, Suzina NE, Bodelier PLE, Liesack W, Dedysh SN (2011) Acetate utilization as a survival strategy of peat-inhabiting *Methylocystis* spp. *Environmental Microbiology Reports*, **3**, 36–46.

Bender M, Conrad R (1992) Kinetics of CH_4 oxidation in oxic soils exposed to ambient air or high CH_4 mixing ratios. *FEMS Microbiology Letters*, **101**, 261–270.

Bodelier PLE (2011) Interactions between nitrogenous fertilizers and methane cycling in wetland and upland soils. *Current Opinion Environmental Sustainability*, **3**, 379–388.

Bodelier PLE, Laanbroek HJ (2004) Nitrogen as a regulatory factor of methane oxidation in soils and sediments. *FEMS Microbiology Ecology*, **47**, 265–277.

Bodelier PLE, Roslev P, Henckel T, Frenzel P (2000) Stimulation by ammonium-based fertilizers of methane oxidation in soil around rice roots. *Nature*, **403**, 421–424.

Bodrossy L, Stralis-Pavese N, Murrell JC, Radajewski S, Weiharter A, Sessitsch A (2003) Development and validation of a diagnostic microbial microarray for methanotrophs. *Environmental Microbiology*, **5**, 566–582.

Boeckx P, Van Cleemput O, Villaralvo I (1997) Methane oxidation in soils with different textures and land use. *Nutrient Cycling in Agrosystems*, **49**, 91–95.

Bull ID, Parekh NR, Hall GH, Ineson P, Evershed RP (2000) Detection and classification of atmospheric methane oxidizing bacteria in soil. *Nature*, **405**, 175–178.

Development Core Team R (2012) *R: A Language and Statistical Computing Environment*, 2.15.1. R Foundation for Statistical Computing, Vienna, Austria.

Diacono M, Montemurro F (2010) Long-term effects of organic amendments on soil fertility. A review. *Agronomy for Sustainable Development*, **30**, 401–422.

Fierer N, Jackson JA, Vilgalys R, Jackson RB (2005) Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. *Applied and Environmental Microbiology*, **71**, 4117–4120.

Gray ND, McCann CM, Christgen B, Ahammad SZ, Roberts JA, Graham DW (2014) Soil geochemistry confines microbial abundances across an artic landscape; implications for net carbon exchange with the atmosphere. *Biogeochemistry*, **120**, 307–317.

Hiltbrunner D, Zimmermann S, Karbin S, Hagedorn F, Niklaus PA (2012) Increasing soil methane sink along a 120-year afforestation chronosequence is driven by soil moisture. *Global Change Biology*, **18**, 3664–3671.

Ho A, Lüke C, Cao Z, Frenzel P (2011a) Ageing well: methane oxidation and methane oxidizing bacteria along a chronosequence of 2000 years. *Environmental Microbiology Reports*, **3**, 738–743.

Ho A, Lüke C, Frenzel P (2011b) Recovery of methanotrophs from disturbance: population dynamics, evenness and functioning. *ISME Journal*, **5**, 750–758.

Ho A, Erens H, Mujinya BB *et al.* (2013a) Termites facilitate methane oxidation and shape the methanotrophic community. *Applied and Environmental Microbiology*, **79**, 7234–7240.

Ho A, Vlaeminck SE, Ettwig KF, Schneider B, Frenzel P, Boon N (2013b) Revisiting methanotrophic communities in sewage treatment plants. *Applied and Environmental Microbiology*, **79**, 2841–2846.

Ho A, Kerckhof F-M, Lüke C, Reim A, Krause S, Boon N, Bodelier PLE (2013c) Conceptualizing functional traits and ecological characteristics of methane-oxidizing bacteria as life strategies. *Environmental Microbiology Reports*, **5**, 335–345.

Ho A, El-Hawwary A, Kim SY, Meima-Franke M, Bodelier P (2015) Manure-associated stimulation of soil-borne methanogenic activity in agricultural soils. *Biology and Fertility of Soils*, **51**, 511–516.

Horz HP, Raghubanshi AS, Heyer J, Kammann C, Conrad R, Dunfield PF (2002) Activity and community structure of methane-oxidising bacteria in a wet meadow soil. *FEMS Microbiology Ecology*, **41**, 247–257.

Horz HP, Rich V, Avrahami S, Bohannan BJM (2005) Methane-oxidizing bacteria in a Californian upland grassland soil: diversity and response to simulated global change. *Applied Environmental Microbiology*, **71**, 2642–2652.

Im J, Lee SW, Yoon S, DiSpirito AA, Semrau JD (2011) Characterization of a novel facultative Methylocystis species capable of growth on methane, acetate and ethanol. *Environmental Microbiology Reports*, **3**, 174–181.

IPCC (2013) Summary for policymakers. In: *Climate Change 2011: Synthesis Report. Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (eds Core Writing Team, Pachauri RK, Meyer L), pp. 5–39. Cambridge University Press, Cambridge.

Kai FM, Tyler SC, Randerson JT, Blake DR (2011) Reduced methane growth rate explained by decreased Northern Hemisphere microbial sources. *Nature*, **476**, 194–197.

Keller M, Mitre ME, Stallard RF (1990) Consumption of atmospheric methane in soils of central Panama: effects of agricultural development. *Global Biogeochemical Cycles*, **4**, 21–27.

Knief C, Lipski A, Dunfield P (2003) Diversity and activity of methanotrophic bacteria in different upland soils. *Applied and Environmental Microbiology*, **69**, 6703–6714.

Kolb S (2009) The quest for atmospheric methane oxidizers in forest soils. *Environmental Microbiology Reports*, **1**, 336–346.

Kolb S, Knief C, Stubner S, Conrad R (2003) Quantitative detection of methanotrophs in soil by novel *pmoA*-targeting real-time PCR assays. *Applied and Environmental Microbiology*, **69**, 2423–2429.

Kolb S, Knief C, Dunfield PF, Conrad R (2005) Abundance and activity of uncultured methanotrophic bacteria involved in the consumption of atmospheric methane in two forest soils. *Environmental Microbiology*, **7**, 1150–1161.

Lee HJ, Kim SY, Kim PJ, Madsen EL, Jeon CO (2014) Methane emission and dynamics of methanotrophic and methanogenic communities in a flooded rice field ecosystem. *FEMS Microbiology Ecology*, **88**, 195–212.

Leng L, Chang J, Geng K, Lu Y, Ma K (2015) Uncultivated *Methylocystis* species in paddy soil include facultative methanotrophs that utilize acetate. *Microbial Ecology*. doi: 10.1007/s00248-014-0540-0

Levine UY, Teal TK, Robertson GP, Schmidt TM (2011) Agriculture's impact on microbial diversity and associated fluxes of carbon dioxide and methane. *ISME Journal*, **5**, 1683–1691.

Lüke C, Frenzel P, Ho A *et al*. (2014) Macroecology of methane-oxidizing bacteria: the *β*-diversity of *pmoA* genotypes in tropical and subtropical rice paddies. *Environmental Microbiology*, **16**, 72–83.

Menyailo OV, Hungate BA, Abraham WR, Conrad R (2008) Changing land use reduces soil CH_4 uptake by altering biomass and activity but not composition of high-affinity methanotrophs. *Global Change Biology*, **14**, 2405–2419.

Mosier AR, Delgado JA (1997) Methane and nitrous oxide fluxes in grasslands in western Puerto Rico. *Chemosphere*, **35**, 2059–2082.

Nazaries L, Tate KR, Ross DJ *et al*. (2011) Response of methanotrophic communities to afforestation and reforestation in New Zealand. *ISME Journal*, **5**, 1832–1836.

Nisbet EG, Dlugokencky EJ, Bousquet P (2014) Methane on the rise – again. *Science*, **343**, 493–495.

Noll M, Frenzel P, Conrad R (2008) Selective stimulation of type I methanotrophs in a rice paddy soil by urea fertilization revealed by RNA-based stable isotope probing. *FEMS Microbiology Ecology*, **65**, 125–132.

Oksanen J, Blanchet F, Kindt R *et al*. (2015) Vegan: community ecology package. R package version 2.2-1, Available at: http://cran.r-project.org (accessed 25 May 2015).

Pratscher J, Dumont MG, Conrad R (2011) Assimilation of acetate by the putative atmospheric methane oxidizers belonging to the USC*α* clade. *Environmental Microbiology*, **13**, 2692–2701.

Priemé A, Christensen S, Dobbie KE, Smith KA (1997) Slow increase in rate of methane oxidation in soils with time following land use change from arable agriculture to woodland. *Soil Biology and Biochemistry*, **29**, 1269–1273.

Rahman MT, Crombie A, Chen Y *et al*. (2011) Environmental distribution and abundance of the facultative methanotroph *Methylocella*. *ISME Journal*, **5**, 1061–1066.

Roslev P, Iversen N, Henriksen K (1997) Oxidation and assimilation of atmospheric methane by soil methane oxidizers. *Applied and Environmental Microbiology*, **63**, 874–880.

Schnell S, King GM (1994) Mechanistic analysis of ammonium inhibition of atmospheric methane consumption in forest soils. *Applied and Environmental Microbiology*, **60**, 3514–3521.

Semrau JD, DiSoirito AA, Yoon S (2010) Methanotrophs and copper. *FEMS Microbiology Reviews*, **34**, 496–531.

Shrestha PM, Kammann C, Lenhart K, Dam B, Liesack W (2012) Linking activity, composition and seasonal dynamics of atmospheric methane oxidizers in a meadow soil. *ISME Journal*, **6**, 1115–1126.

Singh BK, Tate KR, Ross DJ *et al.* (2009) Soil methane oxidation and methanotroph responses to afforestation of pastures with *Pinus radiata* stands. *Soil Biology Biochemistry*, **41**, 2196–2205.

Singh BK, Bardgett RD, Smith P, Reay DS (2010) Microorganisms and climate change: terrestrial feedbacks and mitigation options. *Nature Reviews Microbiology*, **8**, 779–790.

Steenbergh AK, Meima MM, Kamst M, Bodelier PLE (2010) Biphasic kinetics of a methanotrophic community is a combination of growth and increased activity per cell. *FEMS Microbiology Ecology*, **71**, 12–22.

Tate KR (2015) Soil methane oxidation and land-use change – from process to mitigation. *Soil Biology Biochemistry*, **80**, 260–272.

Thangarajan R, Bolan NS, Tian G, Naidu R, Kunhikrishnan A (2013) Role of organic amendment application on greenhouse has emission from soil. *Science of the Total Environment*, **465**, 72–96.

Veraart AJ, Steenbergh AK, Ho A, Kim SY, Bodelier PLE (2015) Beyond nitrogen: the importance of phosphorus for CH_4 oxidation in soils and sediments. *Geoderma*, In press. doi: 10.1016/j.geoderma.2015.03.025.

Warnes GR, Bolker B, Bonebakker L *et al*. (2013) gplots: Various R programming tools for plotting data. R package version 2.12.1, Available at: http://CRAN.R-project.org/package=gplots.

Werling BP, Dickson TL, Isaacs R *et al*. (2014) Perennial grasslands enhance biodiversity and multiple ecosystem services in bioenergy landscapes. *Proceedings of the National Academy of Science of the United States of America*, **111**, 1652–1657.

Yimga MT, Dunfield PF, Ricke P, Heyer J, Liesack W (2003) Wide distribution of a novel *pmoA*-like gene copy among type II methanotrophs, and its expression in *Methylocystis* strain SC2. *Applied and Environmental Microbiology*, **69**, 5593–5602.

Yoshida N, Iguchi H, Yurimoto H, Murakami A, Sakai Y (2014) Aquatic plant surface as a niche for methanotrophs. *Frontiers Microbiology*, **5**, e30. doi: 10.3389/fmicb.2014.00030

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Changes in soil NO_x (a,b), NH_4^+ (c,d), and PO_4^{-3} (e,f) concentrations (mean ± SD; $n = 3$) during incubation in the un-amended and residue-amended sandy loam (a,c,e) and clay (b,d,f) soils.

Figure S2. Potential methane oxidation rate determined in incubations under near atmospheric (20 ppm_v) till high (> 10 000 ppm_v) methane concentrations in the sandy loam and clay soil (mean ± SD; $n = 3$).

Figure S3. Cluster analysis of the standardized microarray data showing primer bias, and the overlaying methanotrophic community composition in both soils using the methanotroph-specific primer pair (A189f/T7_A650r).

Figure S4. Correspondence analysis showing the response of *pmoA* and 16s rRNA gene abundances to environmental variables (CH_4 flux, CO_2 flux, time, and NH_4^+ concentration) at a significant level ($P = 0.025$).

Figure S5. Carbon dioxide flux in un-amended sandy loam (a) and clay (b) soils, and after amendments with sewage sludge, aquatic plant material, compost, wood material, and compressed beet leaves (mean ± SD; $n = 6$).