



Research Paper

The intrinsic methane mitigation potential and associated microbes add product value to compost

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ABSTRACT

Conventional agricultural activity reduces the uptake of the potent greenhouse gas methane by agricultural soils. However, the recently observed improved methane uptake capacity of agricultural soils after compost application is promising but needs mechanistic understanding. In this study, the methane uptake potential and microbiomes involved in methane cycling were assessed in green compost and household-compost with and without pre-digestion. In bottle incubations of different composts with both high and near-atmospheric methane concentrations ($\sim 10,000$ & ~ 10 ppm_v, respectively), green compost showed the highest potential methane uptake rates (up to 305.19 ± 94.43 nmol h⁻¹ g dw compost⁻¹ and 25.19 ± 6.75 pmol h⁻¹ g dw compost⁻¹, respectively). 16S, *pmoA* and *mcrA* amplicon sequencing revealed that its methanotrophic and methanogenic communities were dominated by type 1b methanotrophs, and more specifically by *Methylocaldum szegediense* and other *Methylocaldum* species, and *Methanosarcina* species, respectively. Ordination analyses showed that the abundance of type 1b methanotrophic bacteria was the main steering factor of the intrinsic methane uptake rates of composts, whilst the ammonium content was the main limiting factor, being most apparent in household composts. These results emphasize the potential of compost to contribute to methane mitigation, providing added value to compost as a product for industrial, commercial, governmental and public interests relevant to waste management. Compost could serve as a vector for the introduction of active methanotrophic bacteria in agricultural soils, potentially improving the methane uptake potential of agricultural soils and contributing to global methane mitigation, which should be the focus of future research.

1. Introduction

Methane (CH₄) is Earth's second-most important greenhouse gas (GHG) after CO₂, and the current atmospheric CH₄ concentration of 1.896 parts per million (ppm_v) is the highest in at least 800,000 years (IPCC, 2021). Since the beginning of the industrial revolution in 1750, the atmospheric methane concentration has increased by 156%, and in recent years the increase has even accelerated (Fletcher & Schaefer, 2019; IPCC, 2021). Strong, rapid and sustained reductions in methane emissions are thus needed to limit its impact on global warming (IPCC, 2021). Considering the global methane budget, soil methane uptake is as yet the only known biological sink for atmospheric methane (Saunio et al., 2020). Concurrently, it is estimated that approximately 30% to

50% of yearly methane emissions originate from agricultural practices (Saunio et al., 2020; Tian et al., 2016). However, agricultural soils are often overlooked for their potential to mitigate GHG emissions because both the methane consumption as well as the diversity of the bacteria performing this process are inhibited as a result of conventional agricultural activities like ploughing and the use of mineral fertilizers (Levine et al., 2011). The latter is probably the reason why in climate-smart sustainable agriculture, which aims to minimize GHG emissions and enhance carbon sequestration while maintaining or even enhancing fertility and productivity of the soil (Paustian et al., 2016), data on methane uptake are missing.

Methanotrophs, or aerobic methane-oxidizing bacteria (MOB), are responsible for the biological uptake of methane in upland soils, and

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belong mainly to the phyla *Proteobacteria*, *Verrucomicrobia*, and, as recently shown, the phylum *Actinobacteria* (Dedysh & Knief, 2018; Knief, 2015; van Spanning et al., 2022). Historically, proteobacterial MOB have been divided into two major groups, type I and type II methanotrophs, respectively affiliated with the classes *Gammaproteobacteria* and *Alphaproteobacteria*, based on distinctive features as the carbon fixation mechanism among others (Bodelier et al., (2019); Guerrero-Cruz et al., 2021). Currently, all MOB are still classified in designated types based on phylogeny of marker genes (Knief, 2015). All MOB harbor a methane monooxygenase enzyme that can be present in particulate or soluble form, encoded for by respectively the *pmoA* and *mmoX* gene, which converts methane into methanol (CH_3OH) as the first step in the methane oxidation metabolic pathway (Chistoserdova et al., 2005; Semrau et al., 2010). MOB have been shown to have both high as well as low affinity for the oxidation of methane (Bender & Conrad, 1992; Cai et al., 2016), and the oxidation of methane at (circum-)atmospheric concentrations of $<40 \text{ ppm}_v$ (Singh et al., 2010) has long been thought to be solely catalyzed by high-affinity methanotrophs. These high-affinity MOB are present in both the *Alphaproteobacteria* and *Gammaproteobacteria*, e.g., in clades like Upland Soil Cluster (USC) α and USC γ , respectively (Dedysh & Knief, 2018; Knief et al., 2003), and did not have cultured representatives until recent years (Pratscher et al., 2018; Tveit et al., 2019). Concurrently, it has been shown that low-affinity methanotrophs, generally only able to oxidize methane at concentrations $>600 \text{ ppm}_v$ (Baani & Liesack, 2008; Knief, 2015; Knief & Dunfield, 2005), are also able to oxidize methane to (sub-) atmospheric concentrations, after being activated by oxidizing high concentrations of methane of $>1\%$ (Cai et al., 2016; Ho et al., 2019; Tveit et al., 2019).

Agricultural practice reduces the methane sink capacity of agricultural soils with a factor 3 to 9 compared to undisturbed, well-aerated upland soils (Ho et al., 2015), which is mainly caused by the destruction of the physical structure of the soil affecting the activity of high-affinity MOB (Hütsch, 1998), and more importantly the use of nitrogen-rich mineral fertilizers, resulting in the competitive inhibition of the methane monooxygenase enzyme by ammonium (NH_4^+) (Bodelier & Steenbergh, 2014; Gullledge et al., 1997; Schnell & King, 1994). However, research in recent years has shown that the methane uptake rate of agricultural soils can be significantly enhanced by application of organic amendments, like compost (Ho et al., 2015, 2017, 2019). The most common types of compost used in the Netherlands are vegetable, garden and fruit (VGF-) compost, made from regular source separated household waste and thus subject to a seasonal variation in the composition of the input material, and green compost, mainly made from green waste such as grass clippings and tree cuttings. Before composting, the organic waste can be anaerobically digested as a pre-treatment. During the decomposition phase of the composting process, organic molecules are broken down due to microbial decomposition, whereafter it is reorganized into stable organic molecules during the maturation phase (Azim et al., 2018; Bernal et al., 2009). On an industrial scale, compost is typically processed using either windrow, aerated static pile, or mechanical in-vessel systems, also known as tunnel composting (Brown et al., 2008; Wang et al., 2020). In windrow composting, organic waste is piled in long narrow rows (windrows) and turned mechanically on a regular basis to improve porosity and oxygen availability, and to compost the material in a homogeneous way (Chen et al., 2014; Wang et al., 2020; Zhu-Barker et al., 2017). In aerated static pile composting systems air is forced through the system to maintain aerobic conditions throughout the pile (Brown et al., 2008; Wang et al., 2020), and in mechanical in-vessel or tunnel composting systems the organic waste is composted in confined vessels (e.g. tunnels) with forced aeration and under strictly regulated conditions (Brown et al., 2008; Wang et al., 2020). The use of different types of organic input material and production methods thus result in different types of compost, all with their respective composition and biogeochemical characteristics (Agnew & Leonard, 2003; Haug, 1993; Wang et al., 2023). After production, the compost matures during the maturation phase on static

piles, during which typically the core of the compost pile turns anaerobic and can reach temperatures up to 60°C due to decomposition and microbial activity, enabling for anaerobic methane production by methanogenic Archaea (Brown et al., 2008; Thummes et al., 2007). This internally produced methane diffuses through the oxic outer layer of the maturation pile to the outside atmosphere, where it could concurrently be oxidized by aerobic MOB residing there (Jäckel et al., 2005).

Recent research comparing compost with other organic residues like sewage sludge, digestates and fresh plant material, has demonstrated that compost is the most efficient amendment to stimulate methane uptake in terms of global warming potential (GWP) (Brenzinger et al., 2018, 2021; Ho et al., 2015, 2019). The use of compost as an organic amendment on agricultural soils thus provides a potent methane mitigation strategy. However, the underlying mechanisms for this organic-residue stimulated atmospheric methane uptake by agricultural soils remain to be elucidated. We hypothesize that the application of compost introduces active MOB originating from the compost in the soil. But, research on methanotrophic community and the intrinsic methane uptake capacity of compost is scarce (Jäckel et al., 2005; Mor et al., 2006), especially on the uptake of atmospheric methane by compost, as most research has focused on the CH_4 emissions during the composting process and from compost piles themselves (as reviewed by Brown et al., 2008; Owen & Silver, 2015; Sánchez et al., 2015; Yasmin et al., 2022). Jäckel et al. (2005) were the first to show intrinsic thermophilic methane oxidation in one type of compost and to quantify an active methanotrophic community in this compost, regulating the net methane emission of compost piles. The bacterial and methanotrophic communities of composts during processing and maturation have been assessed before (Wang et al., 2020; Zhou et al., 2020), but not yet in relation to the type of compost and its intrinsic methane uptake potential.

Therefore, in this study, for the first time, the intrinsic methane uptake potential (1) and methanotrophic and methanogenic community abundance and composition (2) of different types of compost was assessed. To this end, the three most common types of compost in the Netherlands, VGF-compost with and without pre-digestion, and green compost, sampled from 6 representative composting companies in the Netherlands, were selected in consultation with the Dutch Waste Management Association. Furthermore, steering factors of the intrinsic methane uptake potential and methanotrophic community of these compost types were determined. Collectively, these experiments aim to elucidate the underlying driving forces of organic-residue stimulated atmospheric methane uptake in agricultural soils. The results of this study will also offer new insights into the potential of, and add value to, the product compost, not only as an agricultural amendment for soil nutrients, but also as potent methane mitigator when applied to agricultural soils, thereby benefiting industrial, commercial, governmental and public interests relevant to waste management.

2. Material and methods

2.1. Compost description, sampling and storage

In consultation with the Dutch Waste Management Association, the three dominantly used Dutch compost-types (VGF-compost with and without pre-digestion, and green compost), collected from compost maturation piles at six representative composting companies in the Netherlands (Table 1), were selected for analysis. Approximately 0.5 kg compost was taken in quadruplicate from two or three specifically selected compost piles per company, varying in maturation time and/or certification to ensure a broad and diverse selection of composts. Samples were taken from both the outer layer of the pile, hereafter referred to as shell (at approximately 30 cm depth), and the core of the pile (at approximately 150 cm depth), using a gouge. At one composting company, Attero (location Deurne), fresh compost was sampled in quadruplicate directly after the composting process. Sub-samples were stored at -20°C and freeze dried using an Alpha 2–4 LD freeze dryer (Martin

Table 1
Compost sampling information and description.

Compost type	Company	Location	Production method	Sampling date	Pile	Pile age	Certification
VFG-compost with pre-digestion	Attero	Venlo, The Netherlands	Tunnel composting	19–6-2019	pile 1	2 weeks	Class A Agrotop-compost
	Orgaworld	Lelystad, The Netherlands	Tunnel composting	25–6-2019	pile 2	6 months	Class A Agrotop-compost
VFG-compost without pre-digestion	Attero	Deurne, The Netherlands	Tunnel composting	19–6-2019	pile 1	1 month	Class C compost
					pile 2	2 months	Class C compost
	Attero	Maastricht, The Netherlands	Tunnel composting	2–7-2019	fresh	n/a	Class A Agrotop-compost
					pile 1	1 week	Class A Agrotop-compost
Green compost	Van Iersel	Biezenmortel, The Netherlands	Windrow	25–6-2019	pile 2	1 month	Class A Agrotop-compost
					pile 1	1 month	Class B Laco-compost
					pile 2	9 months	Class B Laco-compost
	Den Ouden	Haps, The Netherlands	Windrow	2–7-2019	pile 1	1 month	Vigro compost soil
					pile 2	2 months	Vigro green compost
					pile 3	6 months	Fungal dominant humic compost
					pile 1	2 weeks	RHP-compost
					pile 2	1 month	RHP-compost

Christ, Osterode am Harz, Germany), or oven dried at 40 °C for later molecular and chemical analysis, respectively. The remaining sample was stored at 4 °C overnight for subsequent moisture and organic matter content measurements, and incubations with high and near-atmospheric methane concentrations (~10.000 and ~10 ppm_v, respectively) to determine potential methane oxidation rates.

2.2. Physicochemical properties

The moisture content was measured on the basis of loss of weight after oven-drying ~10 g of compost at 105 °C for 24 h, after which the organic matter content was measured as loss of weight after burning the dried samples in an oven at 430 °C for 24 h. Firstly, the pH was measured in water suspension (1:2.5, w/v) after shaking for 2 h. Next, both the NH₄⁺-N and the sum of NO₂⁻-N and NO₃⁻-N ((NO₂⁻ + NO₃⁻)-N content) were determined colorimetrically in a 1 M KCl (1:5, w/v) extract, using a SEAL QuAatro Segmented Flow Analyser (SFA) (Beun-de Ronde B.V., Abcoude, the Netherlands). To this end, the nitrite reduced from nitrate plus any nitrite was quantitated by a modified Griess-Ilosvay method: NO₂⁻ is treated with a diazotizing reagent (sulfanilamide) in HCl and a coupling reagent (N-(1-naphthyl)-ethylenediamine), after which a reddish-purple azo chromophore forms, that is measured at a wavelength of 520 nm. The automated procedure for the determination of ammonia was based on the Berthelot reaction: ammonia is oxidized to chloramine by hypochlorite (released from sodium dichloroisocyanuric acid), after which a blue-green indophenol compound is formed in the presence of salicylic acid (phenol) in alkaline medium (pH 7). Nitroprusside is added as a reaction catalyst. The absorbance was measured at 660 nm. Secondly, the bioavailable H₂PO₄⁻-P + HPO₄²⁻-P content, hereafter referred to as PO₄³⁻, was determined colorimetrically in a 0.01 M CaCl₂ extract (1:10, w/v) using again the QuAatro SFA system. Orthophosphate forms a yellow-colored complex with molybdenum and antimony in an acid medium (phosphomolybdic acid). After reduction with ascorbic acid, a stable blue-colored phosphomolybdenum complex is formed, of which the absorbance was measured at a wavelength of 880 nm. Furthermore, the bioavailable Al, Co, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, S, and Zn contents were determined in an acidified 0.01 M CaCl₂ (1:10, w/v; 1% v/v HNO₃) extract using an ICP-OES 6500 DUO (Thermo Fisher Scientific), and quantification was done using Qtegra Intelligent Scientific Data Solution (ISDS) Software (version 2.10) (Thermo Fisher Scientific).

2.3. Potential methane oxidation rates

To determine the potential methane uptake rates at high and near-atmospheric concentrations, 4 g of fresh compost was incubated for a period of 20 days in a 120 mL glass serum bottle closed off with a black

butyl rubber stopper (Supelco, Sigma-Aldrich, Zwijndrecht, The Netherlands) at room temperature in the dark with an initial headspace methane concentration of ~10.000 and ~10 ppm_v, respectively. CH₄ headspace concentration was measured every day in each bottle by injecting headspace samples taken with a gastight 250 µL pressure-lock glass syringe (VICI AG International, Schenkon, Switzerland) in an Ultra gas chromatograph (GC) (Interscience, Breda, The Netherlands) equipped with a Flame Ionization Detector (FID) and a Rt-Q-Bond (L 30 m, ID 0.32 mm, df 10 µm; Restek, Interscience) capillary column. Helium was used as a carrier gas, and oven temperature was set at 80 °C. One-point calibrations were performed with certified gas mixtures of 10 ppm_v and 990 ppm_v CH₄ in N₂ (Westfalen, Deventer, The Netherlands). Chromeleon™ Chromatography Data System 7.1 software (Thermo Fisher Scientific, Breda, the Netherlands) was used to process the obtained chromatograms from the GC. The methane uptake rates were determined by linear regression of the methane depletion curves for both methane concentrations ($p \leq 0.05$).

2.4. DNA extraction and qPCR assays

DNA was extracted from approximately 0.5 g of compost using the DNeasy PowerSoil Kit (Qiagen, Venlo, The Netherlands) according to manufacturer's instruction, and the quality and quantity was measured with a NanoDrop One Spectrophotometer (Thermo Fisher Scientific). Extracted DNA was stored at -20 °C till further molecular analyses. Quantitative PCR (qPCR) assays were performed targeting 16S rRNA for Archaea and Bacteria, *pmoA* for methanotrophs, and *mcrA* for methanogens. Each assay was performed in duplicate for each DNA extract, and used primers, primer concentration and PCR profiles are shown in Table S1. Each qPCR reaction (total volume 20 µL) was prepared by a QIAgility PCR setup system (Qiagen) and consisted of 2x SensiFAST SYBR (BIOLINE, Alphen aan den Rijn, The Netherlands) or iTaq Universal SYBR Green Supermix (Bio-Rad Laboratories, Lunteren, The Netherlands), forward and reverse primers (Integrated DNA Technologies, Leuven, Belgium), bovine serum albumin (5 µg µL⁻¹; Invitrogen, Breda, The Netherlands), diluted template DNA (10 ng µL⁻¹), and optionally DNase- and RNase-free water. The complete consistency of the qPCR reactions for every used primer pair are shown in Table S1. Standard curves were obtained using serial 10-fold dilutions of a known amount of plasmid DNA from transformed *Escherichia coli* containing the respective target gene fragment (10⁸–10¹ gene copies) (as previously described by Ho et al., 2017, 2019). The qPCR was performed with a CFX96 Touch Real-Time PCR System (Bio-Rad Laboratories). Negative controls were always run with water instead of template DNA. Amplification efficiencies were between 90.8 and 94.3% (16S), 91.1 and 96.3% (*pmoA* total), 89.5 and 97.9% (*pmoA* type Ib), 81.4 and 85.0% (*pmoA* type II), and 82.3 and 87.6% (*mcrA*), with R² values between

0.990 and 0.994 (16S), 0.993 and 1.000 (*pmoA* total), 0.992 and 0.998 (*pmoA* type Ib), 0.997 and 0.999 (*pmoA* type II), and 0.995 and 0.999 (*mcrA*). Amplicon specificity was inferred from the melt curve.

2.5. 16S rRNA, *pmoA* and *mcrA* gene amplicon sequencing and analysis

DNA of samples from 5 representative compost piles (1 VGF-compost with pre-digestion from Attero, Venlo; 1 VGF-compost without pre-digestion from Attero, Deurne; 2 green composts from Van Iersel, Biezenmortel; 1 green compost from Den Ouden, Haps) were selected for sequencing in consultation with the Dutch Waste Management Association, targeting the V4 region of the 16S SSU rRNA of bacteria using the primer pair 515F/806R (Caporaso et al., 2011), *pmoA* for methanotrophs with a nested multiplex-reverse approach using primer pairs A189F/A682R and subsequently A189F/mb661r-A650R (Deng et al., 2019; Holmes et al., 1995), and *mcrA* for methanogens with the primer pair mlas/*mcrA*-rev (Steinberg & Regan, 2008, 2009), using Illumina MiSeq PE250 (16S) or PE300 (*pmoA* and *mcrA*) sequence analysis. A detailed overview of the primers can be found in Table S2. Further processing of the extracted DNA, library construction, amplification and sequencing was done by the Genome Québec Centre d'expertise et de services (Quebec, Canada). Raw sequences can be found at the European Nucleotide Archive (ENA) under the accession number PRJEB56449 (<https://www.ebi.ac.uk/ena/data/view/PRJEB56449>).

Processing of raw sequencing data was done using the DADA2-plugin in QIIME2 (version 2020.2) (Bolyen et al., 2019; Callahan et al., 2016). Sequences were trimmed using cutadapt (Martin, 2011) and subsequently denoised, dereplicated, and filtered of chimeras using denoise-paired. The plugin classify-sklearn (Pedregosa et al., 2011) was used to classify the taxonomic lineages of the representative amplicon sequence variant (ASV) sequences of each specific gene (Bokulich et al., 2018), based on the SILVA non-redundant Small Subunit rRNA database (version 138.1) for 16S (Quast et al., 2013), and on a custom-built reference database based on NCBI sequences (NCBI Resource Coordinators, 2017) for *pmoA* and *mcrA*, and for *pmoA* complemented with sequences from Knief (2015) (Costa et al., 2022). All representative *pmoA* ASV-sequences were aligned with the sequences of the custom-built reference database for *pmoA* with MAFFT (version 7.505) using -auto (Katoh & Standley, 2013), and taxonomically placed in the reference tree of the database with RAXML (version 8.2.12) using -f v (Stamatakis, 2014), based on the Evolutionary Placement Algorithm (EPA) (Berger et al., 2011). The resulting tree was visualized using iTOL (version 6.5.8) (Letunic & Bork, 2021) and *pmoA* clusters were classified based on the classification of Knief (2015). The ASV tables with the appended taxonomy assignments were converted into BIOM-formatted files and exported (McDonald et al., 2012). All downstream analyses were performed in R (version 4.2.1) (R Core Team, 2020), using the phyloseq package (McMurdie & Holmes, 2013). The number of reads in each sample was normalized using the median sequencing depth, and heatmaps were based on Bray-Curtis distance.

2.6. Statistical analyses

All statistical analyses were done using R (version 4.2.1) (R Core Team, 2020). The mean methane oxidation rates, lag phases, physicochemical properties, and abundance of the different targeted genes were tested for normality by Shapiro-Wilk test and for homogeneity of variance by Levene's test. Compost type, part of the pile (shell/core) and company effects, and differences between means were assessed using Scheirer-Ray-Hare test followed by Dunn's *post-hoc* test.

Ordination analyses were performed using the *vegan* package in R (Oksanen et al., 2022). To determine controlling factors of methane uptake rates a redundancy analysis (RDA) was performed, using the variables compost type, part (shell or core), age (storage time on compost pile), all log copy numbers for 16S, total *pmoA*, type Ib *pmoA*, type II *pmoA*, and *mcrA*, and all available physicochemical variables. To

analyze methanotrophic community composition-environment relationships on the species level, an initial detrended correspondence analysis (DCA) revealed a DCA1 axis length of 2.43, indicating a linear community response, and thus a subsequent RDA was performed, using the variables compost type, part, age, and all available physicochemical variables on the bacterial community on species-level. The significance of the environmental variables was tested by a Monte Carlo permutation test (999 unrestricted permutations).

3. Results

3.1. Physicochemical properties

The full quantitative results of the physicochemical analysis can be found in Table S3. The full descriptive statistics can be found in Table S4 for pairwise comparison of compost types, and in Table S5 for pairwise comparison of compost companies. There were no differences in organic matter content between the three types of compost due to large variability between and within compost companies, but green compost had a higher gravimetric moisture content than both VGF-composts ($p \leq 0.001$ for both). Shell samples had a lower gravimetric moisture content than core samples ($p \leq 0.01$). VGF-compost with pre-digestion had the highest pH ($p \leq 0.001$ for both), being slightly basic, and VGF-compost without pre-digestion was on average slightly acidic, whereas green compost was generally neutral (Table S3).

VGF-compost without pre-digestion had the highest NH_4^+ and PO_4^{3-} content, followed by VGF-compost with pre-digestion, and green compost had the lowest NH_4^+ and PO_4^{3-} content (all $p \leq 0.001$). ($\text{NO}_2^- + \text{NO}_3^-$)-N content varied greatly between composts of all types, between and within individual piles, and was even undetectable (<0.4 mg/kg) in some piles. Green compost had the highest ($\text{NO}_2^- + \text{NO}_3^-$)-N content compared to VGF-compost with and without pre-digestion ($p \leq 0.01$ and $p \leq 0.05$, respectively). Shell samples had a higher ($\text{NO}_2^- + \text{NO}_3^-$)-N content than core samples ($p \leq 0.05$), whilst there were no differences in NH_4^+ and PO_4^{3-} content between core and shell samples (Table S3).

Additionally, VGF-compost without pre-digestion had the highest bioavailable Co, Cu, K, Mg, P, S, and Zn content, whilst green compost had the lowest bioavailable Al, Cu, Co, Fe, K, Mo, Na, Ni, P, S, and Zn content, and VGF-compost with pre-digestion had the lowest bioavailable Mn content (see Table S4 for levels of significance) (Table S3). Shell samples had a higher bioavailable S content than core samples ($p \leq 0.05$), while core samples had a higher bioavailable Mn content than shell samples ($p \leq 0.001$), and there was no difference in the bioavailable content of the other chemical elements between core and shell samples (Table S3).

3.2. Potential methane oxidation rates

Comparing the three compost types, green compost showed the highest potential methane uptake rates (up to 305.19 ± 94.43 nmol h^{-1} g dw compost $^{-1}$) and shortest lag phase, i.e., the time it takes for the sample to show methane uptake activity, in the incubation with high methane concentration ($\sim 10,000$ ppm $_v$) (see Table S4 for levels of significance) (Fig. 1ab), although there was a distinct variability between the composts of the two green compost companies, as green compost from Van Iersel had a higher potential methane uptake rate and shorter lag phase than green compost from Den Ouden ($p \leq 0.001$) (Table S5). VGF-compost with pre-digestion showed the second-highest potential methane uptake rates and second-shortest lag phase, whilst VGF-compost without pre-digestion showed no activity at all during the 20-day incubation period (Table S4). Only green compost oxidized methane to sub-atmospheric concentrations (<1.89 ppm $_v$) during the incubation period, whereas both VGF-compost with and without pre-digestion did not oxidize methane to concentrations below 10 ppm $_v$ (Table S6). There were no differences in the potential methane uptake rates and lag phase between shell and core samples.

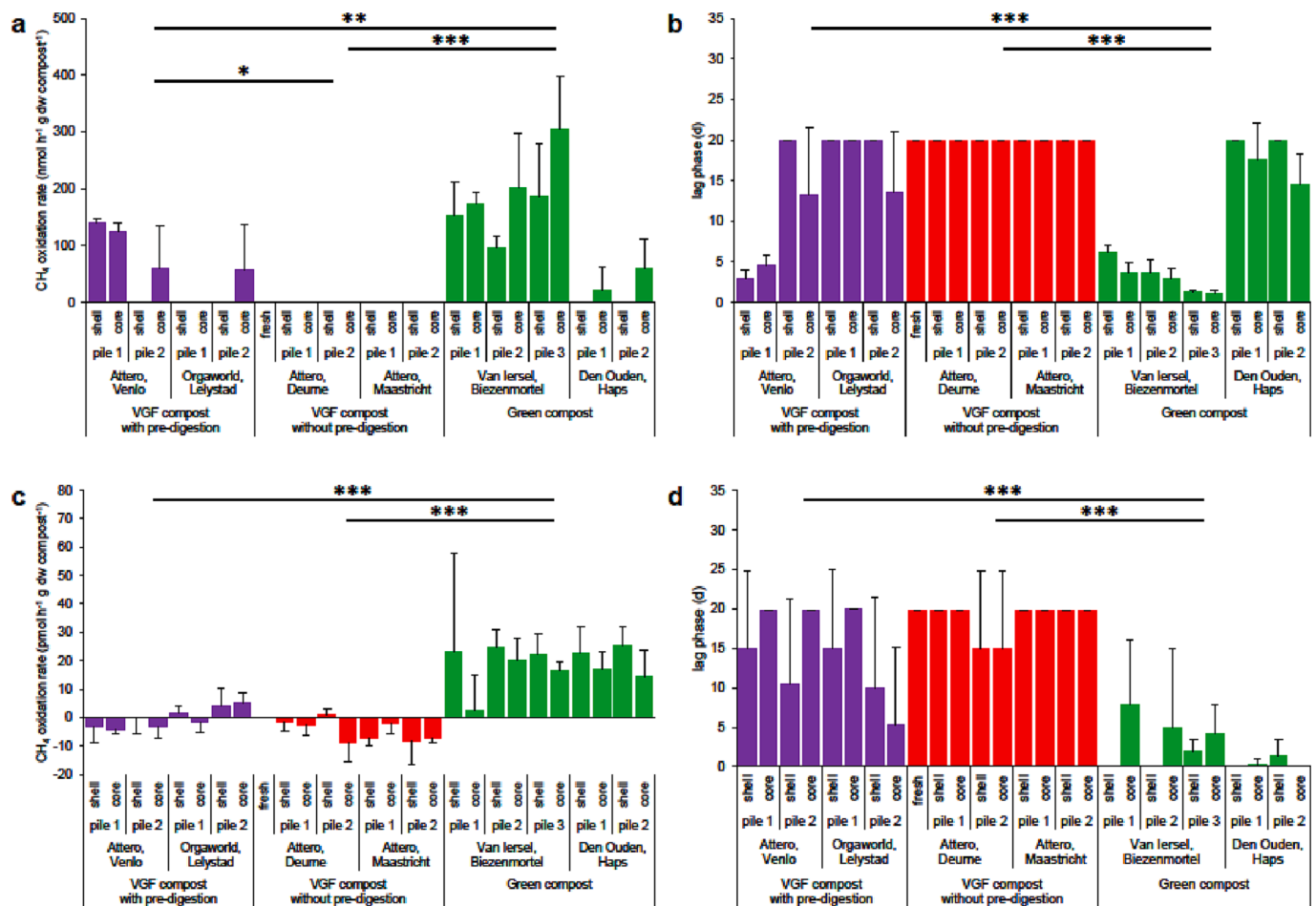


Fig. 1. Potential methane uptake rates (a, c) and lag phases (b, d) of composts in incubations with $\sim 10,000$ ppm_v (a, b) and ~ 10 ppm_v (c, d) methane (mean $\pm$ SD; $n = 4$). The level of significance between compost types is indicated by an asterisk (Dunn's test; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Full descriptive statistics are available in [Table S4](#).

In the incubation with near-atmospheric methane concentration (~ 10 ppm_v), green compost again showed the highest potential methane uptake rates (up to 25.19 ± 6.75 pmol h⁻¹ g dw compost⁻¹) and shortest lag phase (see [Table S4](#) for levels of significance) (Fig. 1cd), but in this incubation, green compost of both companies showed similar potential methane uptake rates and lag phases. Both VGF-compost with and without pre-digestion showed similar low to negative (i.e., emission) methane uptake rates and high lag phases. Only green compost oxidized methane to sub-atmospheric concentrations during the incubation period in a limited number of individual replicates ([Table S6](#)). There were again no differences in the potential methane uptake rates and lag phases between shell and core samples in the incubation with near-atmospheric methane concentrations.

3.3. Abundance analyses of relevant microbial groups

VGF-compost with pre-digestion had the highest total copy number of bacterial 16S rRNA genes per gram dry weight compost. VGF-compost without pre-digestion had the second-highest and green compost the lowest total 16S copy number (see [Table S4](#) for levels of significance) (Fig. 2a), and there were no differences in 16S copy number between core and shell samples.

Green compost did however have the highest total *pmoA* copy number, having ~ 50 x and ~ 300 x more *pmoA* copies per gram dry weight compost than VGF-compost with and without pre-digestion respectively ($p \leq 0.001$ for both). Green compost also had the highest type Ib *pmoA* copy number, having ~ 20 x and ~ 200 x more type Ib *pmoA*

copies per gram dry weight compost than VGF-compost with and without pre-digestion respectively ($p \leq 0.001$ for both). There were no differences in total *pmoA* and type Ib *pmoA* copy number between shell and core samples. Interestingly, there were no differences between compost types in the total number of type II *pmoA* copy numbers, but shell samples did have a higher type II *pmoA* copy number than core samples ($p \leq 0.05$) (Fig. 2bcd).

For *mcrA*, green compost had the highest total copy number, having ~ 10 x more *mcrA* copies per gram dry weight compost than both VGF-compost types ($p \leq 0.001$ for both), and core samples had a higher *mcrA* copy number than shell samples ($p \leq 0.01$) (Fig. 2e).

3.4. Bacterial, methanotrophic, and methanogenic community analysis

The composition of the total bacterial community of composts was determined using amplicon sequencing analysis of the 16S rRNA gene, and a total of 2605 ASV's were identified and classified. VGF-compost with and without pre-digestion was mainly dominated by *Firmicutes* and *Actinobacteria*, whereas in the bacterial community of green composts there is a clear increase in abundance of *Acidobacteria*, *Chloroflexi*, and *Proteobacteria*, the phylum containing the proteobacterial methanotrophic lineages. This is also reflected in the beta-diversity plot, where the three types of compost are well-separated. Furthermore, the alpha-diversity is highest for green composts, and fairly comparable for both VGF-composts with and without pre-digestion (Fig. S1).

The methanotrophic community was determined on basis of amplicon sequencing analysis of *pmoA*, and a total of 227 ASV's were

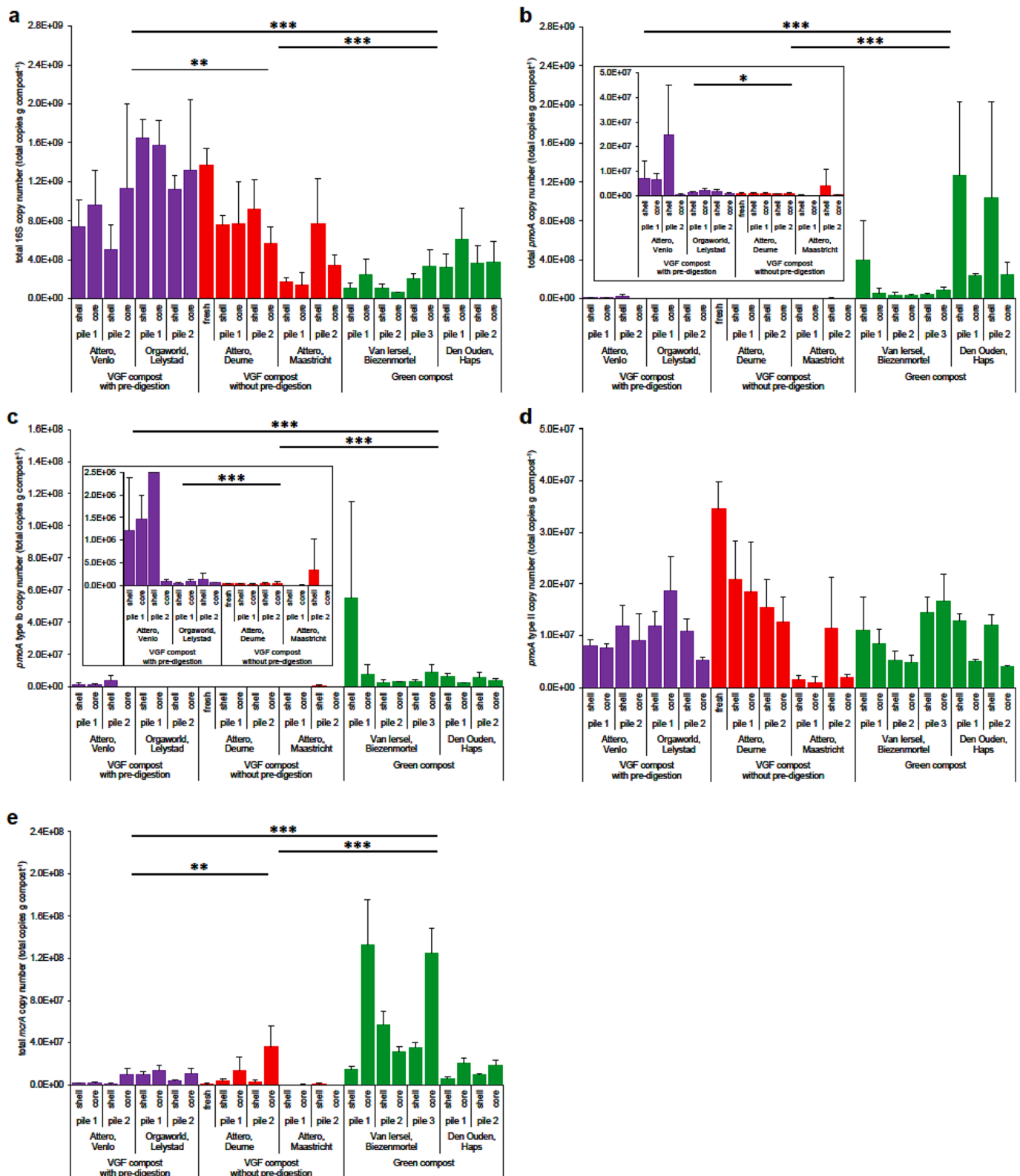


Fig. 2. Abundance of 16S (a), total *pmoA* (b), *pmoA* type Ib (c), *pmoA* type II (d), and *mcrA* (e) genes per gram dry weight of compost. In (b) and (c) the insets are zoomed in parts of the respective graphs (mean $\pm$ SD; n = 4). The level of significance between compost types is indicated by an asterisk (Dunn's test; * p < 0.05; ** p < 0.01; *** p < 0.001). Full descriptive statistics are available in Table S4.

identified and classified on basis of a custom-built reference database (Table S7). VGF-compost with pre-digestion is dominated by *Methylocaldum marinum* in shell samples and by *Methylocystis hirsuta* in core samples. The methanotrophic community composition of VGF-compost

without pre-digestion is less pronounced as there are no clear dominating species and no clear differences between shell and core samples, and the dominant genera are *Methyloparacoccus* and *Methylocaldum*. Green composts from both sampled companies have an almost identical,

distinct methanotrophic community, which is hyper-dominated by *Methylocaldum*-like methanotrophs. In some individual replicates the relative abundance of ASV001 (*Methylocaldum*-like) was 100% of the total. The amplicon sequencing targeting the 16S rRNA revealed that this *Methylocaldum*-like sequence (ASV001) is likely affiliated to *Methylocaldum szegediense*, which was highly dominant in the green compost samples. The fungal dominant humic compost is also dominated by ASV001, and by *Methylocaldum tepidum* and *Methylocystis parvus* (Fig. 3a). The alpha-diversity of the *pmoA* ASV's is highest in green compost, albeit with great variation between the composts of the two companies, followed by VGF-compost with pre-digestion and VGF-compost without pre-digestion has the lowest alpha diversity (Fig. 3b). The NMDS plot calculated on basis of the Bray-Curtis distance, indicating the beta-diversity, shows a clear separation of the three types of compost (Fig. 3c). The hyper-dominance of ASV001 is also reflected in the heatmap of abundant ASV's. Type Ib methanotrophic ASV's classified as *Methyloparacoccus murrellii* (ASV005), *Methylocaldum tepidum* (ASV002 & ASV032), *Methylocaldum marinum* (ASV004, ASV008 & ASV009), and *Methylocaldum szegediense* (classified as *Methylocaldum*-like based on *pmoA*) (ASV001) dominate all composts, except for the core samples of VGF-compost with pre-digestion, which is mainly dominated by type II methanotrophic ASV's classified as *Methylocystis hirsuta* (ASV003, ASV007 & ASV019) and *Methylocystis* sp. (ASV011) (Fig. 3d).

The taxonomic placement of the methanotrophic ASV's in the reference tree confirmed the taxonomic classification of these sequences by the plugin classify-sklearn. A total of 124 unique ASV's were taxonomically placed within the type IIa methanotrophs and 1 ASV within the type IIb methanotrophs, confirming its classification as a Cluster_4 USCA-related methanotroph. A total of 96 unique ASV's were placed within type Ib methanotrophs, 5 ASV's were placed within type Ic methanotrophs, and 1 ASV was placed within type Ia methanotrophs (Fig. 4).

The methanogenic community composition was determined using amplicon sequencing analysis targeting the *mcrA* gene, and a total of 552 *mcrA* ASV's were identified and classified. All composts were dominated by the genus *Methanosarcina*, with *Methanosarcina horonobensis* being the dominant species. Other methanogenic genera present in all composts are *Methanothermobacter*, *Methanobacterium* and *Methanomassiliicoccus*. *Methanocella* is only found in green compost, and *Methanosarcina mazei* has only been identified in VGF-compost with pre-digestion. There is a notable higher relative abundance of *Methanobacterium* in core samples compared to shell samples, which is most pronounced in green composts. VGF-compost with pre-digestion has the highest alpha-diversity, and the alpha-diversity of the green composts is variable between the two companies. All three types of compost have distinct methanogenic communities, as they are well-separated in the beta-diversity plot (Fig. S2).

3.5. Controlling factors of methane oxidation

An RDA was performed to explore possible controlling factors of the methane uptake rates and lag phases of the different compost types in both the incubations with high and near-atmospheric methane concentrations. It resulted in RDA1 and RDA2 axes with eigenvalues of 1.55 and 0.43 and proportions of explained variance of 0.518 and 0.144, respectively, for a combined explained variation of 66.14%. A permutation test (999 permutations) revealed significant correlations for the variables compost type ($p = 0.001$), age ($p = 0.005$), organic matter content ($p = 0.007$), moisture content ($p = 0.009$), type Ib *pmoA* copy number ($p = 0.004$), NH_4^+ content ($p = 0.049$) and Mg content ($p = 0.044$), and moderate significant correlations for part, total *pmoA*, type II *pmoA*, and *mcrA* copy number ($0.05 \leq p \leq 0.10$) (Fig. 5a). The RDA plot revealed that a high methane uptake rate and a short lag phase are associated with green compost, and correlate greatly with high type Ib *pmoA* and *mcrA* copy numbers ($R^2 = 0.34$ and 0.11 , respectively), and have limited correlation with a high moisture content ($R^2 = 0.03$) and

type II *pmoA* copy number ($R^2 < 0.01$). Negative correlations were observed with a high organic matter content ($R^2 = 0.14$), and with ammonium and magnesium content ($R^2 = 0.02$ and 0.01 , respectively).

3.6. Controlling factors of the methanotrophic community

To analyze methanotrophic community composition-environment correlations and explaining variables, an RDA on the abundances of the methanotrophic community was carried out. For the species-environment correlations, the RDA1 and RDA2 axes had eigenvalues of 18.11 and 14.60, and explained proportions of variance of 0.294 and 0.237, respectively, for a combined explained variation of 53.18%. A permutation test (999 permutations) revealed significant correlations for the methanotrophic community composition with the variables compost type ($p = 0.001$), age ($p = 0.001$), organic matter content ($p = 0.010$), and PO_4^{3-} content ($p = 0.001$), and moderate significant correlations for aluminum, cobalt, iron, molybdenum, and zinc content ($0.05 \leq p \leq 0.10$) (Fig. 5b). On the species-level, *Methylocaldum szegediense* and *Methylothermus thermalis* were associated with green compost, and *Methylocaldum marinum*, *Methyloparacoccus murrellii*, *Methylocaldum tepidum*, and *Methylocystis hirsuta* were associated with both VGF-composts. *Methylocystis parvus*, *Methylocystis hirsuta*, *Methylocaldum tepidum*, and *Methylosinus trichosporium* correlated with a longer storage time on the pile and higher organic matter content, whilst *Methylocaldum marinum* and *Methyloparacoccus murrellii* correlated with a short storage time of the pile and lower organic matter content. *Methylocaldum marinum* and *Methyloparacoccus murrellii* were strongly associated with a high phosphate content (Fig. 5b).

For the family-environment correlations, the RDA1 and RDA2 axes had eigenvalues of 9.21 and 1.59, and explained proportions of variance of 0.584 and 0.101, respectively, for a combined explained variation of 68.56%. A permutation test (999 permutations) revealed significant correlations for the methanotrophic community composition with the variables compost type ($p = 0.045$), age ($p = 0.001$), organic matter content ($p = 0.007$) (Fig. 5c). On the family-level, *Methylothermaceae* and *Methylococcaceae* correlated with green compost, whilst *Methylocystaceae* correlated with both VGF composts. *Methylocystaceae* were associated with a longer storage time on the compost pile, whereas for *Methylothermaceae* the opposite was true, and the abundance of *Methylococcaceae* was not influenced by storage time on the pile. Furthermore, *Methylothermaceae* and *Methylocystaceae* were associated with a higher organic matter content, and *Methylocystaceae* correlated with a high phosphate content (Fig. 5c).

4. Discussion

4.1. The intrinsic methane mitigation potential of different compost types

In this study, the intrinsic methane uptake potential and the methanotrophic community composition of three types of compost was assessed, together with potential steering physicochemical factors. For both incubations with $\sim 10,000$ and ~ 10 ppm_v methane, green compost had the highest intrinsic potential methane uptake rates and shortest lag phase of the three compost types, although there is a substantial variation between the green composts of the two different companies in the $\sim 10,000$ ppm_v methane incubation (Fig. 1ac & Table S5). Only very limited research determining the intrinsic methane mitigation potential of composts has been published before. Overall, the highest intrinsic methane mitigation potential of green compost of this study is in agreement with earlier research by Mor et al. (2006), where two VGF-composts and three green composts were tested on their methane uptake potential, and only two green composts showed significant methane oxidation activity in incubations with $\sim 50,000$ ppm_v ($\sim 5\%$) methane. Furthermore, it has been found that green garden waste compost showed the highest methane oxidation rates in batch incubations with $\sim 13\%$ of methane (Pedersen et al., 2011). Jäkel et al. (2005) were the first to

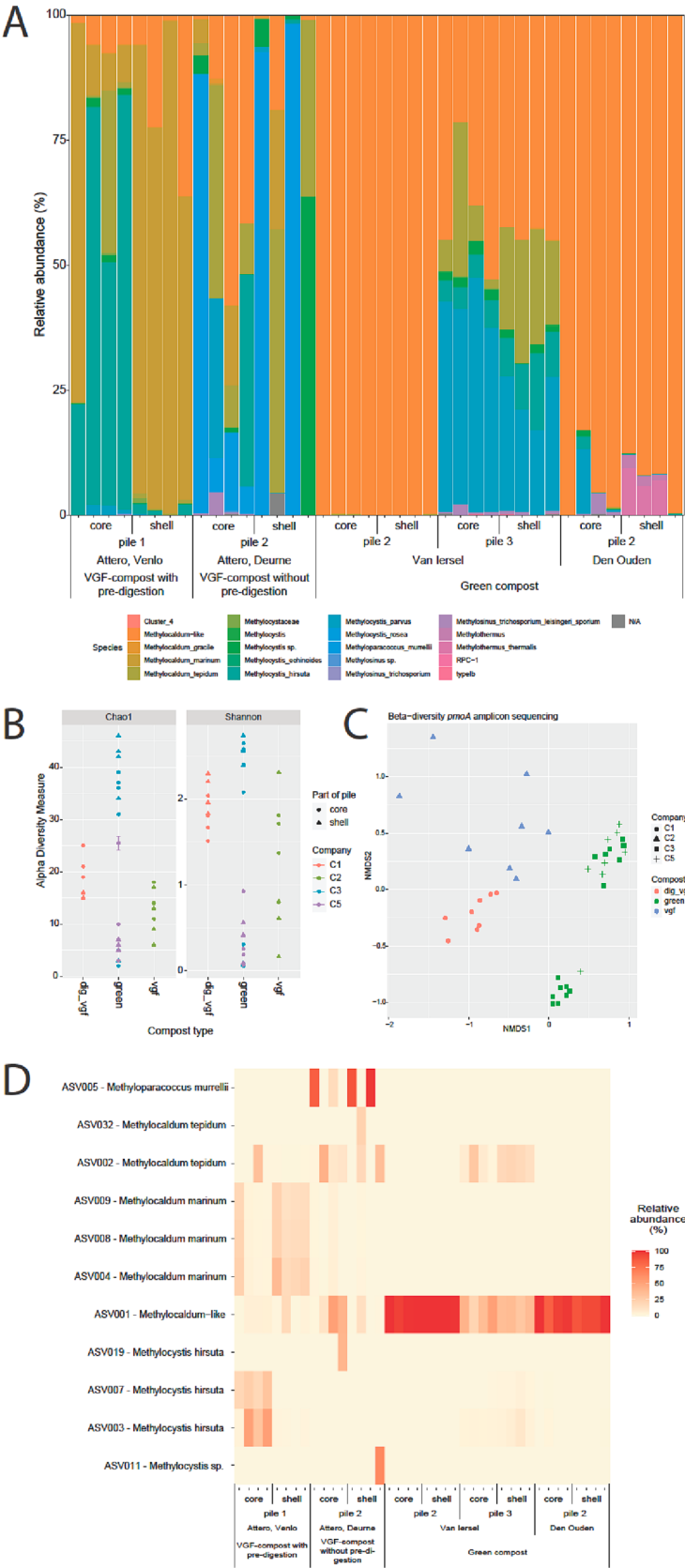


Fig. 3. Abundance barplot of methanotrophic community composition on species level (a), alpha-diversity based on both Chao1 and Shannon diversity indices (b), beta-diversity by NMDS on basis of Bray-Curtis distance (c) and heatmap based on Bray-curtis distance of ASV's with an abundance of at least 20% in one sample (d), based on *pmoA* ASV's. For alpha-diversity, composts sorted by type and colored by company: VGF-compost with pre-digestion (dig_vgf) of Attero, Venlo (C1), green compost (green) of Van Iersel, Biezenmortel (C3) and Den Ouden, Haps (C5), and VGF-compost without pre-digestion (vgf) of Attero, Deurne (C2). For beta-diversity, composts colored by type and shaped by company. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

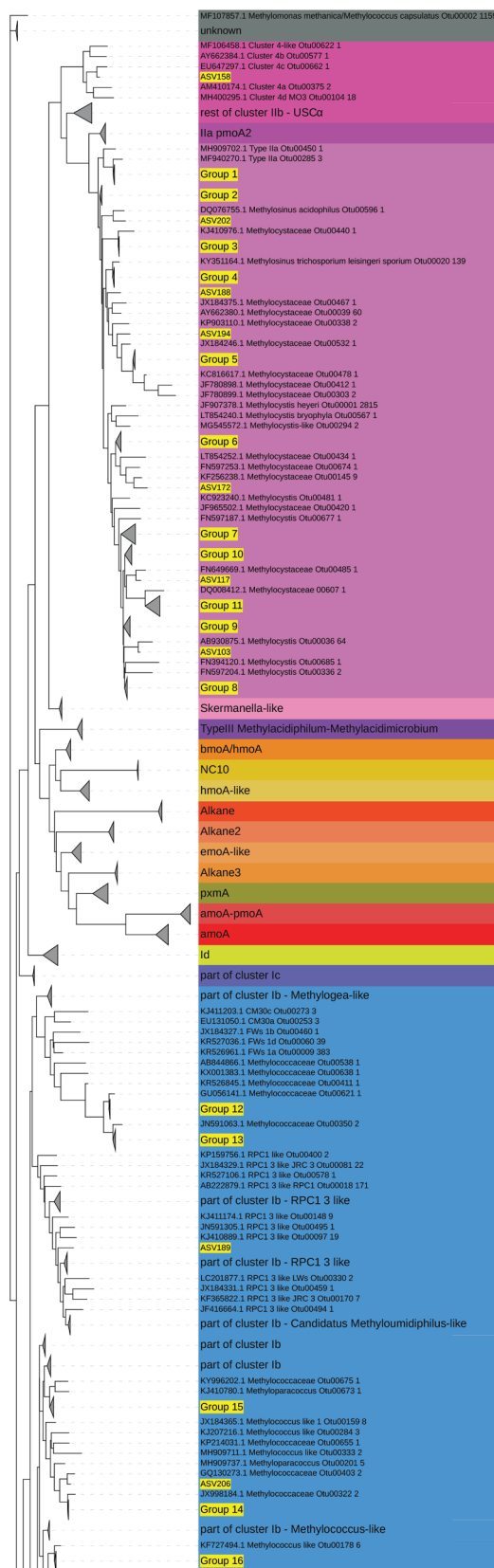


Fig. 4. Taxonomic placement of methanotrophic *pmoA* ASV's in the reference tree. Clustering and respective coloring based on Knief (2015). ASV's with the same taxonomical placement are grouped, a full list of ASV's, their taxonomy and their grouping, is available in Table S7. A full un-collapsed tree is available as Fig. S3.

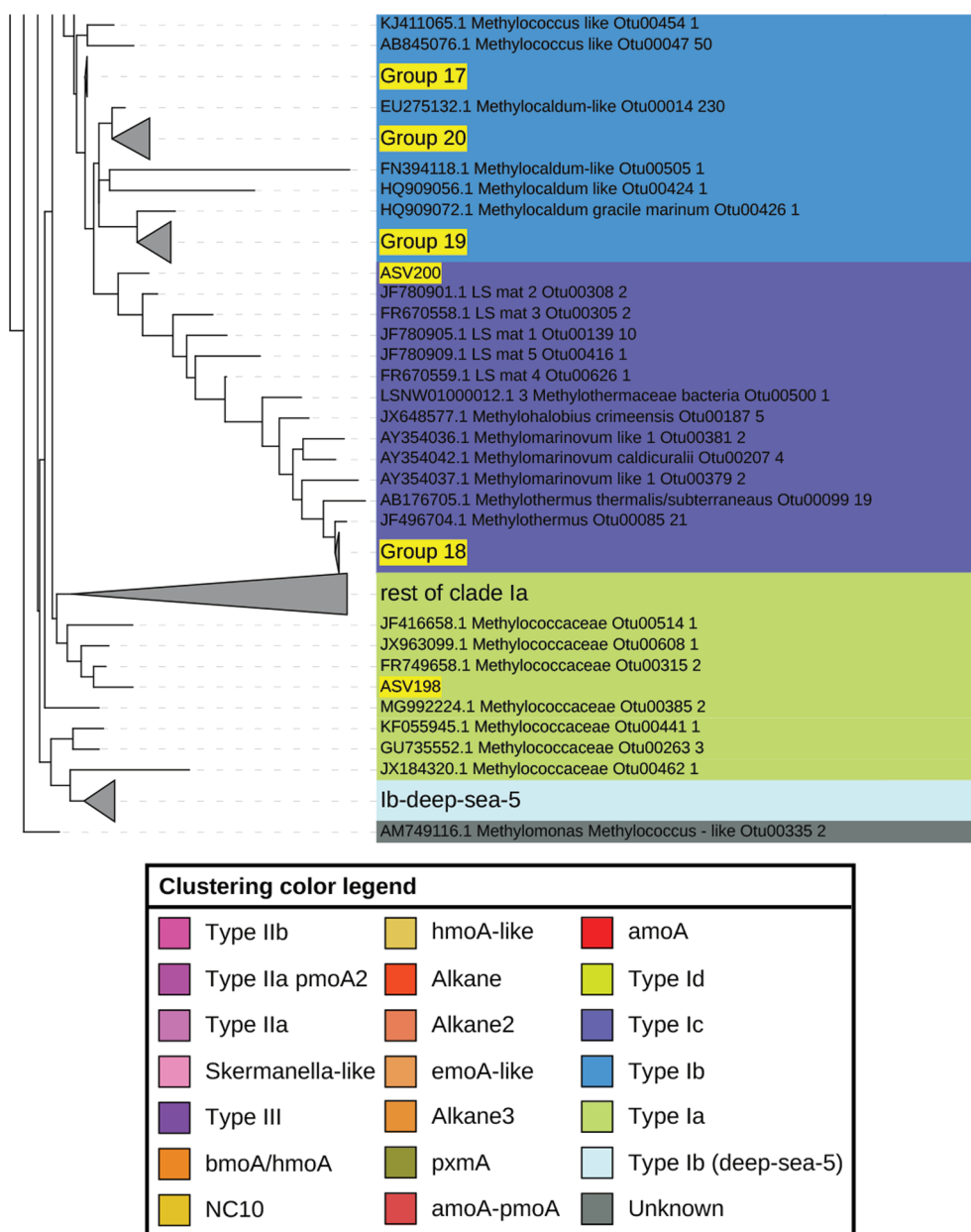


Fig. 4. (continued).

report a potential for aerobic methane oxidation in compost, found in incubations of green compost (70% organic material of communal bio-waste and 30% loppings from tree-cuttings) with ~20.000 ppm_v (~2%) methane at 50 °C. These studies reported average and peak uptake rates of approximately 2- to 20-fold higher than the highest uptake rates found in this study, which can be explained by the higher initial methane concentrations in the incubations. Altogether, it can thus be concluded that green compost has a higher intrinsic methane oxidation potential than VGF-compost (with or without pre-digestion). In this study it has now been demonstrated for the first time that green compost is able to oxidize methane at near-atmospheric concentrations (~10 ppm_v), and is even able to oxidize methane to sub-atmospheric concentrations (Table S6), emphasizing the potential of green compost to mitigate atmospheric methane.

4.2. The effect of the physicochemical composition of compost on the intrinsic methane mitigation potential

The physicochemical analysis of the three types of compost showed that there is a great variation in their chemical composition (Table S3). First and foremost, green compost has the lowest concentrations for most of the measured nutrients and elements. This can be explained as household and food waste, used to produce VGF-compost, typically contain high concentrations of salts and nutrients, especially compared to the more recalcitrant green waste used to produce green compost. Most importantly, VGF-composts have a significantly higher ammonium (NH₄⁺) concentration than green compost, which is a well-known competitive inhibitor of the methane monooxygenase enzyme, thus reducing the methane oxidation potential of the compost (Bodelier & Steenbergh, 2014; Gullede et al., 1997; Schnell & King, 1994). This is confirmed by the ordination analysis exploring the possible controlling factors of the methane oxidation potential, as the ammonium content is one of the strongest negatively contributing factors to the variation

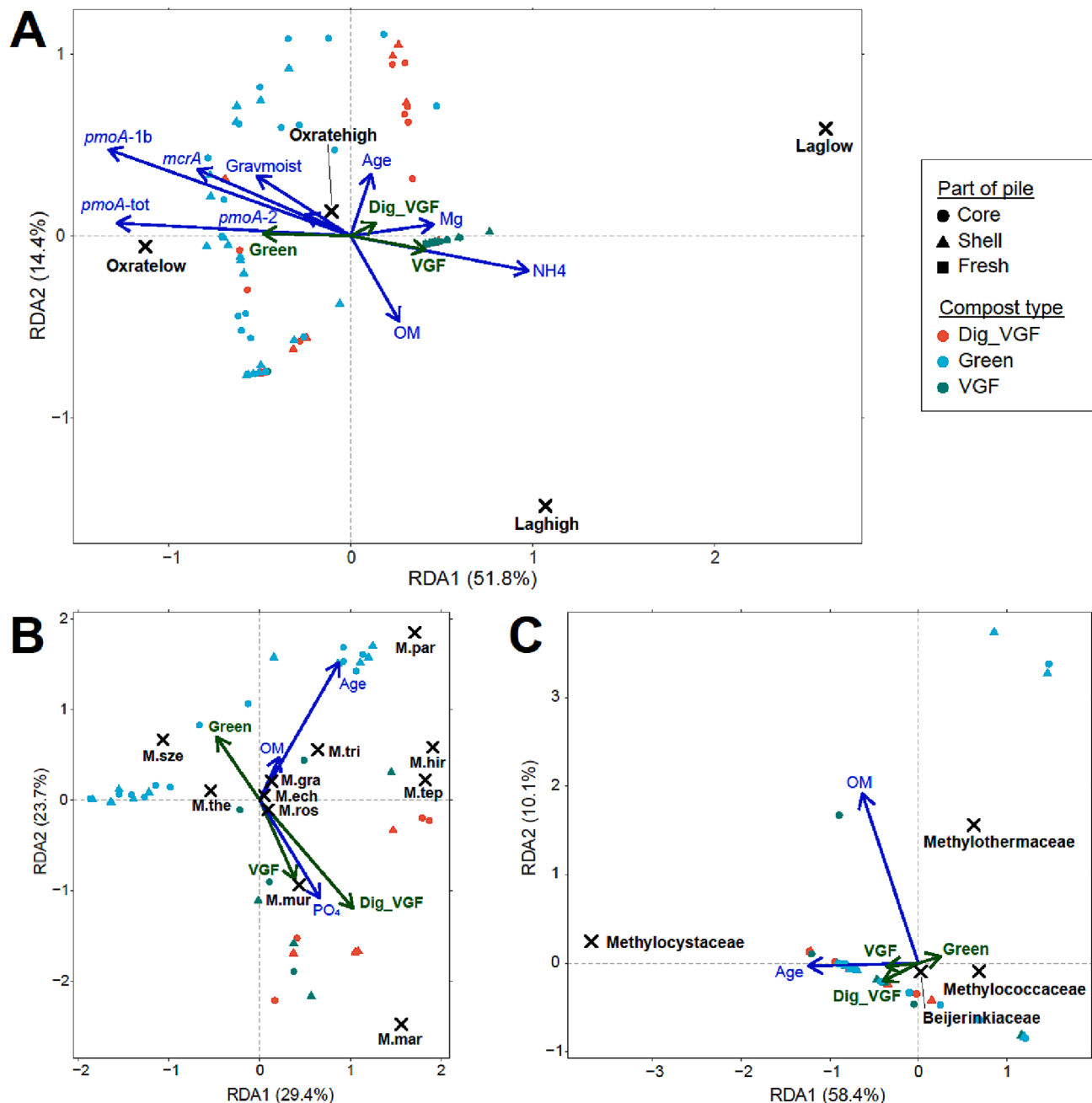


Fig. 5. RDA on variables explaining the variability observed in methane oxidation rates and lag phases (a), in the methanotrophic community-environment on both the species-level (b), and the family-level (c) in compost, obtained using methane uptake rates in incubations with $\sim 10,000$ ppm_v CH₄ (Oxratehigh) and ~ 10 ppm_v CH₄ (Oxratelow) and lag phases in the respective incubations (Laghigh and Laglow, respectively), methanotrophic species abundance of *Methylocaldum szegediense* (M.sze), *Methylocaldum marinum* (M.mar), *Methylocaldum tepidum* (M.tep), *Methylocaldum gracile* (M.gra), *Methylothermus thermalis* (M.the), *Methyloparacoccus murrellii* (M.mur), *Methylocystis hirsuta* (M.hir), *Methylocystis parvus* (M.par), *Methylocystis rosea* (M.ros), *Methylocystis echinoides* (M.ech), *Methylosinus trichosporium* (M.tri), the methanotrophic family abundance of *Methylococcaceae*, *Methylothermaceae*, *Methylocystaceae*, and *Beijerinckiaceae*, and the environmental variables type of compost (Dig_VGF for VGF-compost with pre-digestion, VGF for VGF-compost without pre-digestion, Green for green compost), storage time on pile (Age), organic matter content (OM), moisture content (Gravmoist), total *pmoA* copy number (*pmoA*-tot), type Ib *pmoA* copy number (*pmoA*-1b), type II *pmoA* copy number (*pmoA*-2), total *mcrA* copy number (*mcrA*), ammonium content (NH₄), magnesium content (Mg) and phosphate content (PO₄). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

observed in the methane oxidation rates in incubations with both high and near-atmospheric concentrations of methane (Fig. 5a). The high ammonium content in VGF-composts thus appears to be the main factor limiting the uptake of (near-)atmospheric methane concentrations. Concurrently, the organic matter content was also identified as a negative contributor to the methane uptake potential of composts. Although this is in accordance with a large-scale study on compost properties impacting the methane oxidation efficiency (Huber-Humer

et al., 2011), this negative relationship between organic matter content in compost and its intrinsic methane mitigation potential is noteworthy, particularly given the known positive association between methane uptake and organic management in arable soils (Ho et al., 2015; Skinner et al., 2014). This inverse relationship is probably due to a high positive co-correlation of organic matter content and ammonium content, as overall, in this study, composts with a higher organic matter content correlate significantly with a higher ammonium content ($p \leq 0.05$).

Interestingly, when only considering the green composts used in this study, there is a significant positive correlation between the organic matter content and methane uptake ($p \leq 0.05$), and for the VGF-composts, both with and without pre-digestion combined, the opposite is true ($p \leq 0.001$), emphasizing that the identification of the organic matter content as a negative contributor to the intrinsic methane uptake potential is due to the organic matter content co-correlating with the ammonium content, as the input material used to produce VGF-composts is often rich in both nitrogen and carbon (Brown et al., 2008; Wang et al., 2023).

In another study, Huber-Humer et al. (2011) found that the nutrient (total N and total P) and the organic matter content were the main steering factors of the methane oxidation capacity of compost materials, and thus not the C:N ratio. The nutrient content and C:N ratio of composts from the same composting companies as used in this study were determined by Clocchiatti et al. (2020), and are similar to the composts from Huber-Humer et al. (2011), suggesting that for the composts used in this study, the C:N ratio is also not a steering factor of the methane oxidation efficiency. The observed differences in moisture and ($\text{NO}_2^- + \text{NO}_3^-$)-N content between shell and core samples are due to the fact that the shell samples are exposed to the air, enabling evaporation and allowing aerobic metabolic processes as nitrification to take place.

4.3. Methanogen and methanotroph abundance and dynamics

The abundance analyses showed that green compost has the highest *mcrA* copy number (Fig. 2e), and the amplicon sequencing analysis revealed a dominance of *Methanosarcina horonobensis* in all composts (Fig. S2a). The methanogenic genus *Methanosarcina* has been found dominant in compost in other studies (Thummes et al., 2007; Wen et al., 2021; Zhang et al., 2021). *Methanosarcina* species are considered robust methanogens (de Vrieze et al., 2012), because of their moderately thermophilic nature and great adaptability to new conditions (Conklin et al., 2006; Shimizu et al., 2011), explaining the dominant abundance of *Methanosarcina* in the composts assessed in this study. The abundance analyses also showed that green compost both has the lowest total 16S copy number (Fig. 2a) and the highest total *pmoA* copy number (Fig. 2b), indicating a high relative abundance of methanotrophs in green compost, which is also being reflected in a higher relative abundance of methanotrophic and proteobacterial ASV's on basis of 16S rRNA amplicon sequencing (Fig. S1a). The assays specifically targeting type Ib and type II methanotrophs show that the higher total *pmoA* copy number in green compost is solely caused by type Ib methanotroph abundance (Fig. 2c), as there are no differences in type II *pmoA* copy number between the VGF-composts and green compost (Fig. 2d). This is in line with the methanotrophic community analysis on basis of *pmoA* amplicon sequencing, showing a hyper-dominance of *Methylocaldum* species, especially *Methylocaldum szegediense*, in green compost. The ordination analyses on the methanotrophic community confirmed the association of *Methylocaldum szegediense* (Fig. 5b) and *Methylococcaceae* (Fig. 5c) with green compost. Furthermore, the ordination analyses show that type II methanotrophs of the family *Methylocystaceae* and the species *Methylocystis parvus* and *Methylocystis hirsuta* are most strongly correlated with a longer storage time on the pile, whereas the families *Methylococcaceae* and *Methylothermaceae* and thermophilic species *Methylocaldum marinum*, *Methylocaldum szegediense*, *Methylothermus thermalis*, and *Methyloparacoccus murrellii* (Bodrossy et al., 1997; Hoefman et al., 2014; Trotsenko et al., 2009), are strongly correlated with a short storage time on the pile, indicating a community shift during the maturation of the compost on the pile. The aforementioned thermophilic methanotrophic species are dominating the methanotrophic community in the compost after the thermophilic phase of the composting procedure, and the longer the storage time on the pile, the more the methanotrophic community shifts to a dominance of more mesophilic type II methanotrophs of the family *Methylocystaceae*. Altogether, it can be concluded that green compost has a distinct methanotrophic and

bacterial community, which is also reflected in the beta-diversity plots (Fig. 3c & Fig. S1c), that is dominated by thermophilic type Ib methanotrophic bacteria, more specifically *Methylocaldum* species from the family *Methylococcaceae*.

Interestingly, in the previously mentioned study of Jäckel et al. (2005), the first to report a potential for aerobic methane oxidation in compost, one methanotrophic strain was isolated from the compost, that was identified as *Methylocaldum szegediense*. The thermophilic optimum temperature of 55 °C for growth and methane oxidation of *Methylocaldum szegediense* was suggested for favoring the development of a stable community of *Methylocaldum szegediense* during the thermophilic phase of compost production. Multiple other studies also reported the dominance of type I(b) methanotrophs in compost, among others identified via PLFA analysis, DGGE, 16S MiSeq- and pyrosequencing, and *pmoA* MiSeq sequencing (Chen et al., 2014; Halet et al., 2006; Liu et al., 2023; Wilshusen et al., 2004; Zhang et al., 2021, respectively), all concluding that the type I methanotrophs are mainly responsible for the methane oxidation activity peaks of compost and are most important in determining the net surface emissions of methane. This is in accordance with the ordination analysis exploring possible controlling factors of methane oxidation in this study, which also indicates the importance of the abundance of type Ib methanotrophs (Fig. 5a). Furthermore, the abundance of methanogens was identified as an important possible controlling factor (Fig. 5a), agreeing with conclusions from earlier research that the dynamics between methanotrophic bacteria and methanogenic archaea is a determining factor of the net methane emission of composts (Chen et al., 2014; He et al., 2019; Sharma et al., 2011).

4.4. Activity of methanotrophic bacteria at (sub-)atmospheric methane concentrations

However, as yet no *Methylocaldum* species or even type Ib methanotrophs have been demonstrated to be able to oxidize methane at (near-)atmospheric concentrations (Knief, 2015). The type-strain *Methylocaldum* sp. E10a even loses methane oxidation activity when incubated at ≤ 1000 ppm, methane. Concurrently, methanotrophic type-strains from the genus *Methylocystis* have been demonstrated to be able to oxidize methane at (sub-)atmospheric concentrations (Knief & Dunfield, 2005). The question which MOB are responsible for the methane oxidation to sub-atmospheric and at near-atmospheric methane concentrations is thus very relevant. In soil systems, it has been shown that atmospheric methane oxidation is mainly driven by high-affinity MOB from the clusters USC α and USC γ (Deng et al., 2019; Täumer et al., 2021; Wang et al., 2022), whilst it also has been shown that under high methane conditions the methanotrophic community in soils is dominated by *Methylocaldum* (Wang et al., 2022). In this study, only one ASV was identified in the high-affinity MOB cluster, ASV158, classified as a Cluster-4 type IIb methanotroph (Table S7), taxonomically placed most closely to the USC α -cluster (Fig. 4). However, as this ASV was only found in one individual sample, its abundance is too low to be of any explanatory value for the observed near- to sub-atmospheric methane oxidation. Secondly, in soil systems it has been shown before that conventional MOB are capable of oxidizing methane to (sub-)atmospheric concentrations after being activated by high concentrations of methane (Cai et al., 2016; Ho et al., 2015, 2019). These high methane concentration spikes can gear up the enzyme machinery of the low-affinity MOB, and the MOB can potentially store energy derived from the oxidation of high concentrations of methane in polyhydroxyalkanoate storage compounds such as polyhydroxybutyrate (PHB) (Cai et al., 2016; Ho et al., 2013; Mason-Jones et al., 2021), which can be subsequently used to oxidize methane at low concentrations. Previously, it has been shown that part of the PHB cycle was upregulated in the transcriptomic analysis of high-affinity methane oxidation after high methane concentration spikes (Cai et al., 2016). During the maturation phase of compost on a pile, the pore oxygen concentration in

the core of a compost pile maintains steadily at low (<5%) but non-zero concentrations during maturation (Zeng et al., 2018) as a result of the decomposition of the organic material and microbial respiration, a sufficiently low oxygen concentration for internal methane production by methanogenic Archaea. The methane production in the core of the compost pile can result in methane concentrations of up to 60 to 70% (Brown et al., 2008), sufficient to potentially activate the methanotrophic community on the oxic-anoxic interface in the maturing compost pile (Cai et al., 2016; Ge et al., 2014; Ho et al., 2013; Reim et al., 2012; Zeng et al., 2018), as is also suggested by Fig. 5a.

The hyper-dominance of type Ib MOB and more specifically *Methylocaldum* species in the green composts that show near- to sub-atmospheric methane oxidation in the incubations in this study, suggest that these conventional, low-affinity type Ib methanotrophs are responsible for the observed methane oxidation, potentially by being activated by high concentrations of methane that are produced during the composting process and maturation phase of the compost. Apparently, these MOB have the ability to utilize methane over a wide concentration range, as was observed in a recently described study (Tveit et al., 2019).

4.5. Compost as a vector for the introduction of active methanotrophic bacteria to agricultural soils

It has been shown in previous research that compost and manure can serve as an inoculum for methanogenic species in arable soil (Gattinger et al., 2007; Thummes et al., 2007). This suggests that compost can also serve as a vector for the introduction of methanotrophs when applied to agricultural soils. However, it remains to be elucidated whether, and if so, for how long, the methanotrophic community of composts remains active when applied to agricultural soils and introduced in the native methanotrophic community. Ho et al. (2015) showed that the use of compost as an organic amendment to agricultural soils can offset up to ~16% of net emitted carbon dioxide, which can counteract the reduction of the methane sink capacity of agricultural soils with a factor 3 to 9 compared to undisturbed, well-aerated upland soils (Ho et al., 2015; Levine et al., 2011). Taken together with this, the results of this study emphasize the potential of composts, and especially green compost, as a methane mitigation strategy. The high intrinsic methane uptake rates at near-atmospheric methane concentration that were shown by green compost, suggest that the application of compost as an organic amendment to agricultural soils can be a viable addition to the soil's methane uptake potential, and thereby creating added value to (green) compost as a product. Moreover, recent research has shown that there is a potential for the engineering of organic amendments like composts, modifying them towards desired effects (Chavez-Rico et al., 2023), suggesting that composts could also be engineered to maximize their intrinsic methane mitigation potential.

The results provide pivotal new insights on the mechanisms of this organic amendment stimulated methane uptake capacity improvement, as composts can serve as a vector for the introduction of active methanotrophic bacteria in agricultural soils, and the composts analyzed in this study were colonized by distinct and active methanotrophic communities.

4.6. Limitations and future recommendations

The selection of compost types, composting companies, and compost piles was carefully done in consultation with the Dutch Waste Management Association. Nevertheless, the research in this study has thus far only been performed on a selection of representative composts used in the Netherlands, and as such, the results of this study should be carefully extrapolated to broader conclusions. It should also be noted that, as discussed in the introduction, compost is a product with a seasonally varying composition. However, the results of this study show very clear characteristics specific to different compost types, allowing

for realistic generalizations and interpretations that will be beneficial to industrial, commercial, governmental and public interests in waste management. Altogether the results of this study provide added value to compost as a product.

Future research should focus on the application of these results in the field. Does the use of composts as an organic amendment improve the methane uptake potential of agricultural soils, and what are the underlying mechanisms hereof? Also, it could be tested whether composts can be engineered towards a desired physicochemical and methanotrophic community composition, or whether the microbes with the right properties are already present in the compost. Composts with these desired properties can be prioritized for more sustainable application.

5. Conclusions

For the first time, the intrinsic methane mitigation potential and methanotrophic community of different types of compost has been assessed this extensively. Green compost showed the highest methane uptake potential and methane uptake activity up to sub-atmospheric concentrations. Green compost also had the highest abundance of methanotrophic bacteria, and its methanotrophic community was dominated by type Ib methanotrophs of the family *Methylococcaceae* and more specifically of the genus *Methylocaldum*. The abundance of (type Ib) methanotrophs was the main steering factor of the methane uptake potential of composts, whereas ammonium and organic matter content were the main limiting factors. The results suggest that the conventional type Ib methanotrophic bacteria dominating the green compost are responsible for the observed near- to sub-atmospheric methane oxidation, as the methanotrophic community can be activated during the compost production and maturation phase, where sufficient high methane concentrations are produced. However, it remains to be elucidated whether the methanotrophic community of composts remains active when applied to agricultural soils.

The results of this study add value to compost as a product, as it proves to be a potent methane mitigator. These new relevant insights benefit industrial, commercial, governmental and public interests relevant to waste management.

CRedit authorship contribution statement

Stijn G. van den Bergh: Conceptualization, Methodology, Software, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Iris Chardon:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – review & editing. **Marion Meima-Franke:** Methodology, Investigation. **Ohana Y. A. Costa:** Methodology, Software. **Gerard W. Korthals:** Resources, Funding acquisition. **Wietse de Boer:** Conceptualization, Writing – review & editing, Supervision. **Paul L.E. Bodelier:** Conceptualization, Methodology, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

CRedit author contribution based on Brand et al. (2015). All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.wasman.2023.07.027>.

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