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Divergent drivers of the microbial methane sink in temperate forest and grassland soils

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Abstract

Aerated topsoils are important sinks for atmospheric methane (CH_4) via oxidation by CH_4-oxidizing bacteria (MOB). However, intensified management of grasslands and forests may reduce the CH_4 sink capacity of soils. We investigated the influence of grassland land-use intensity (150 sites) and forest management type (149 sites) on potential atmospheric CH_4 oxidation rates (PMORs) and the abundance and diversity of MOB (with qPCR) in topsoils of three temperate regions in Germany. PMORs measurements in microcosms under defined conditions yielded approximately twice as much CH_4 oxidation in forest than in grassland soils. High land-use intensity of grasslands had a negative effect on PMORs (−40%) in almost all regions and fertilization was the predominant factor of grassland land-use intensity leading to PMOR reduction by 20%. In contrast, forest management did not affect PMORs in forest soils. Upland soil cluster (USC)-α was the dominant group of MOBs in the forests. In contrast, USC-γ was absent in more than half of the forest soils but present in almost all grassland soils. USC-α abundance had a direct positive effect on PMOR in forest, while in grasslands USC-α and USC-γ abundance affected PMOR positively with a more pronounced contribution of USC-γ than USC-α. Soil bulk density negatively influenced PMOR in both forests and grasslands. We further found that the response of the PMORs to pH, soil texture, soil water holding capacity and organic carbon and nitrogen content differ between temperate forest and grassland soils. pH had no direct effects on PMOR, but indirect ones via the MOB abundances, showing a negative effect on USC-α, and a positive on USC-γ abundance. We conclude that reduction in grassland land-use intensity and afforestation has the potential to increase the CH_4 sink function of soils and that different parameters determine the microbial methane sink in forest and grassland soils.

KEYWORDS

greenhouse gas, land-use intensity, methane, methanotrophs, potential methane oxidation rates, soil, Upland soil cluster

1 | INTRODUCTION

The tropospheric concentration of methane (CH_4) has increased by 150% since the beginning of the industrial era and its warming potential is 28 times higher than that of CO_2 (Ciais et al., 2013). More than one-third of global CH_4 emissions derive from methanogenesis in soils under anoxic conditions, which occur, for example, in wet rice cultivation and permanent or temporary wetlands (Ciais et al., 2013; Conrad, 2009). In contrast, well-aerated soils typically function as net sinks for atmospheric CH_4 due to the consumption of CH_4 by methanotrophic bacteria (Kolb, 2009; Le Mer & Roger, 2001; Tate, 2015). CH_4 oxidation is primarily considered to be aerobic and is catalysed by bacteria within the *Alphaproteobacteria*, *Gammaproteobacteria*, and *Verrucomicrobia* but also the anaerobic candidate phylum NC10 (Knief, 2015). The key enzyme for atmospheric methanotrophy is the particulate CH_4 monooxygenase (pMMO; Baani & Liesack, 2008; Knief, 2015). Studies targeting the gene encoding the alpha subunit of pMMO (*pmoA*) as a functional marker have found that CH_4-oxidizing bacteria (MOB) are highly diverse; additionally, several major soil lineages are currently poorly characterized or even missing cultured representatives, such as the Upland soil cluster (USC)-γ (Knief, 2015). Methanotrophs solely dependent on atmospheric CH_4, however, have resisted cultivation until very recently, when the atmospheric CH_4 oxidizer *Methylocapsa gorgona* was isolated (Tveit et al., 2019). *M. gorgona* is a member of USC-α that has been detected in many different soils, such as forest and permafrost soils with mostly neutral to acidic pH (Degelmann et al., 2010; Kolb, 2009; Kolb et al., 2005; Pratscher et al., 2018; Tveit et al., 2019). Other MOB assumed to be involved in atmospheric CH_4 oxidation are members of USC-γ, which was detected in neutral to alkaline upland soils and have recently been identified as the main methanotrophs in alpine grassland soils (Deng et al., 2019; Knief, 2015).

Whether a soil acts as source or sink for CH_4 is strongly controlled by soil environmental parameters such as oxygen, substrate availability, temperature, and N status, all of which are known to change the habitat and living conditions for methanogens as well as for MOB (Bodelier, 2011; Lyu et al., 2018).

Land-use change and management practices influence these soil environmental parameters and may therefore alter soil CH_4 fluxes (Tate, 2015). A recent global meta-analysis revealed that the conversion from a natural to any anthropogenic land use increases CH_4 emissions (McDaniel et al., 2019). However, the effects of land-use intensity and its mediating drivers on CH_4 emissions have not yet been resolved. It is generally assumed that fertilizers, especially ammonium-based fertilizers, decrease CH_4 oxidation rates due to competitive inhibition of the methane monooxygenase. In grassland soils, different management practices and intensities have been shown to influence atmospheric CH_4 uptake. For example, heavy livestock grazing reduces CH_4 uptake by 24%–31% (Chen et al., 2011) and N fertilization can negatively affect CH_4 oxidation in cultivated soils (Mosier et al., 1991). In a more recent study on three Swiss grassland sites with different management intensities and elevations, highest CH_4 uptake was found at the least intensively and lowest CH_4 uptake at the most intensively managed site (Imer et al., 2013). A meta-analysis by Liu and Greaver (2009), which found CH_4 uptake reduced when upland grassland soils were N-fertilized, further indicates that CH_4 uptake by grassland soils can be influenced by land-use intensity.

CH_4 uptake rates by forest soils were typically more pronounced than those of grassland soils with deciduous forests the strongest sinks for atmospheric CH_4 (Degelmann et al., 2009; Liu & Greaver, 2009). Similar to grassland management, forest management also influences atmospheric CH_4 uptake. The conversion of natural hardwood forests to spruce and pine forests reduced its CH_4 sink potential by about two-thirds (Borken et al., 2003; Maurer et al., 2008). Other forest management effects, such as soil disturbance, compaction during clear-cutting and thinning, or N-deposition, have also been found to negatively affect the CH_4 sink function of forest soils (Frey et al., 2011; Steudler et al., 1989; Teepe et al., 2014). However, a general negative effect of N fertilization on CH_4 oxidation in both forest and upland grassland soils has also been questioned as it seems to depend on the amount of N present in soil (Bodelier, 2011; Bodelier & Laanbroek, 2004).

To date, few studies have linked atmospheric CH_4 oxidation to the abundances of the methanotrophic groups and the environmental factors influencing their abundances. It has been found for different soils that the proportion of USC-α was positively correlated with CH_4 uptake (Nazaries et al., 2013) and thus might be a key group of MOB contributing to the global atmospheric CH_4 sink. Malghani et al. (2016) also linked the abundance of USC-α methanotrophs to CH_4 oxidation rates. However, environmental factors can differentially influence CH_4 oxidation and the methanotrophic community. For example, increasing soil moisture has been shown to lower CH_4 oxidation while stimulating MOB abundance in forest soils (Shrestha et al., 2012). Recently, USC-γ has been identified as a dominant group in grassland soils (Zhao et al., 2018), but it is not clear how the abundances of different MOB groups relate to CH_4 oxidation in soils or how they respond to land use and land-use intensity.

To investigate the relationship between MOB abundance, CH_4 oxidation, land-use type (grassland and forest) and intensity of land use in more detail, we sampled topsoils of 150 grassland and 150 forest sites that differ in their grassland land-use intensity and in the type of forest management, respectively, in three temperate regions in Germany (Schwäbische Alb [ALB], Hainich-Dün [HAI], and Schorfheide-Chorin [SCH] region). We measured potential CH_4 oxidation rates, soil physicochemical properties, and determined the abundances of the methanotrophic bacterial groups USC-α and USC-γ, which are assumed to be involved in CH_4 oxidation at atmospheric concentrations. We hypothesized that in grasslands, high management intensity (fertilization and/or frequent grazing and mowing) will reduce CH_4 oxidation rates due to higher availability of ammonium in soils and to greater soil compaction by machinery use and/or livestock trampling. In forests, intense management will reduce CH_4 oxidation rates due to soil compaction resulting from forest machinery. Furthermore, soils with a higher abundance of MOB will have higher potential CH_4 uptake rates. In addition, we assume that soil environmental properties drive both CH_4 uptake and the abundance of MOB in soils.

2 | MATERIALS AND METHODS

2.1 | Experimental design

The study was conducted within the framework of the Biodiversity Exploratories project for long-term functional ecosystem research (Fischer et al., 2010; www.biodiversity-exploratories.de). The Biodiversity Exploratories are located in three different climate regions of Germany: Schwäbische Alb (southwest, annual mean precipitation: 700–1,000 mm, annual mean temperature 6–7°C, abbreviated as ALB), Hainich-Dün (central Germany, annual mean precipitation: 500–800 mm, annual mean temperature 6.5–8°C, abbreviated as HAI), and Schorfheide-Chorin (northeast, annual mean precipitation: 500–600 mm, annual mean temperature 8–8.5°C, abbreviated as SCH). In each region, 50 grassland (50 m × 50 m) and 50 forest sites (100 m × 100 m) were selected (Table S1). Soil types varied between sites and were classified according to WRB (IUSS Working Group WRB, 2015). The grasslands were managed as meadows, pastures, or mown pastures. Grazing intensity, fertilization, and mowing frequency were monitored annually and a land-use intensity index (LUI) was calculated for each site for 2016 (Blüthgen et al., 2012). The LUI was calculated for the year 2016 for each plot as the square root of the sum of the standardized grazing intensity (livestock units days of grazing ha^{-1} $year^{-1}$), mowing frequency per year and the amount of nitrogen applied on the plot per year (kg nitrogen ha^{-1} $year^{-1}$). The values were standardized according to its mean within all plots.

In the forest sites, dominant tree species were beech, spruce, pine, or oak. A forest management index (ForMI) was calculated based on the proportion of non-native tree species, the proportion of harvested tree biomass, and the proportion of dead wood showing signs of saw cuts (Kahl & Bauhus, 2014).

2.2 | Soil sampling and soil properties

All 299 sites were sampled in May 2017. In each plot, one composite soil sample was prepared consisting of 14 soil cores (upper 10 cm of mineral soil) that were taken along two intersecting transects (20 m in grasslands; 40 m in forest). The organic layer (forests) and vegetation above the soil (grasslands) had been removed before sampling. Samples were sieved (<5 mm) and stored at 4°C for measurements of potential CH_4 oxidation and at −20°C for DNA extraction and measurements of soil properties.

Gravimetric soil water content was determined by drying 3–6 g of soil at 105°C to constant weight. Soil pH was measured by mixing 10 g of air-dried sieved soil with 25 ml 0.01 M $CaCl_2$ solution and measuring the pH of the suspension with a glass electrode (pH meter 538 and pH glass electrode SenTix 61; WTW). An aliquot of the soil sample was dried at 105°C to determine the bulk density based on the sample volume and mass. The proportion of sand (2–0.063 mm), silt (0.063–0.002 mm), and clay (<0.002 mm) in the soil samples was determined by sieving and sedimentation (DIN-ISO 11277). Samples for the determination of soil texture were taken in May 2011 as described above. Soil texture was classified according to the German 'Standortserkundungsanweisung' (SEA 1974) with the R package 'soiltexture' (Moeys, 2018). For total carbon and total nitrogen measurements, samples were sieved (<2 mm) and air-dried, ground in a ball mill (RETSCH MM200; Retsch) and analysed in an elemental analyzer (VarioMax) at 1,100°C. Inorganic carbon was determined with the same elemental analyzer after the organic carbon had been removed by combustion of soil samples at 450°C for 16 hr. Organic carbon concentrations were calculated as the difference between total carbon and inorganic carbon. Ammonium (NH_4^+) and nitrate (NO_3^-) were extracted with 0.5 M K_2SO_4 (soil to extractant ratio [w/v] of 1:4), shaken on a horizontal shaker for 30 min at 250 r.p.m., and centrifuged for 30 min at 4,400 *g*. The concentrations of NH_4^+-N and NO_3^--N were measured on an autoanalyzer using UV spectroscopy (Bran & Luebbe).

2.3 | Potential methane oxidation rates

Potential atmospheric CH_4 oxidation rate (PMOR) was measured under atmospheric mixing ratios (2 ppm CH_4) in microcosms of all 299 soil samples in triplicate. For this, an equivalent to 40 g soil dry weight (organic soils) and 70 g (mineral soils) fresh soil was weighed into plastic vessels (average diameter 6.8 cm). The water content was adjusted to 34% of the maximum water holding capacity of the respective soil, since 34% of maximum water holding capacity has been previously identified as the mean optimum for CH_4 oxidation in different soils (Gulledge & Schimel, 1998). The water content was adjusted by gently air-drying the soil at 4°C (for 3–72 hr) or adding deionized water to the soil. The soil was compacted in the plastic vessels to a bulk density of 0.7–0.8 g/cm^3 and pre-incubated at 20°C for 5 days. The plastic vessels with the soils were put into glass jars (500 ml Weck Gläser; J. Weck GmbH u. Co. KG) which were closed with airtight lids and incubated the soil samples at 20°C in the dark. After airtight closing of the microcosms the headspace was over-pressurized by adding 50 ml of ambient air. Gas samples (12 ml) were taken from the headspace immediately, 1, 2 and 6 hr after closing with an airtight syringe through a three-way stopcock and transferred into pre-evacuated exetainers (5.9 ml; Labco Lt). Gas concentrations were measured with an Agilent 7890 gas chromatograph equipped with a flame ionization detector (for CH_4) coupled with a methanizer (for CO_2; Agilent Technologies Inc.). Gas flux rates were calculated by the slope of the regression line of a linear regression of the gas concentration against time.

2.4 | DNA extraction and qPCR

DNA was extracted from the soil (stored at −20°C) with the Qiagen DNeasy PowerSoil Kit according to the manufacturer's instructions, and stored at −20°C until further use. DNA concentrations were measured on a NanoDrop™ 8000 (Thermo Fischer Scientific). A preselection of 30 soils from all regions and land-use types were screened for the presence of *pmoA/mmoX* genes of specific methanotrophic taxa (general *pmoA*, USC-α, USC-γ, Verrucomicrobia, and *Methylocella*; Costello & Lidstrom, 1999; Kolb et al., 2003,

2005; Sharp et al., 2012; Rahman et al., 2011; Table S2). For the preselection, five samples with different PMOR (highest, lowest and from each region and land-use type) were chosen. Three groups of methanotrophic bacteria were quantified with three different quantitative PCR assays in a 7500 Fast Real-Time PCR System (Applied Biosystems). A general *pmoA* assay was used to detect a broad spectrum of MOB (Costello & Lidstrom, 1999), the FOREST assay (Kolb et al., 2003) to quantify USC-α specific *pmoA*, and the GAM assay to amplify a USC-γ specific *pmoA* (Kolb et al., 2005). The qPCRs (20 µl) were performed in 96-well plates with SensiFAST™Sybr Lo-ROX master mix (Bioline [Meridian Life Science], Inc.) using a three-step thermal profile with denaturation at 95°C for 25 s, annealing at assay specific temperature (Table S2) for 20 s, and elongation at 72°C for 45 s. Bovine serum albumin was added to the master mix (final concentration 2 ng/µl).

2.5 | Statistics

All statistical analyses were carried out in R (version 3.5.1; R Core Team, 2018). Data were checked for normal distribution and homogeneity of variance and transformed if necessary. Significant differences between groups were tested with a two-sample *t* test for normally distributed data and a Mann–Whitney test for non-normally distributed data. Linear regression analysis was used to assess the relationship between PMORs and physicochemical and land-use parameters. The significance levels reported were based on Pearson's coefficient. Grasslands were grouped into high and low LUI and into heavily and weakly grazed using the k-means algorithm (Hartigan & Wong, 1979). Since PMORs were region-specific (especially in grasslands), PMORs were normalized to be able to compare the effects of land use among all regions. The PMOR norm was calculated by dividing the PMOR of each plot by the mean PMOR of the respective region. qPCR measurements that were below detection limit were set to 100 for correlation analyses and structural equation modelling (SEM). SEM was used to unravel direct and indirect effects on PMORs. For this, an a priori model was set up. It was hypothesized that soil parameters (bulk density, pH, and sand content) and land-use intensity in forest and grassland have a direct influence on PMORs and also an indirect effect via MOB abundances. Bulk density was chosen as a representative for other soil factors (water holding capacity, organic carbon, and total nitrogen content) with which it covariates strongly. pH was chosen since it is an important factor for microbial activity (Lauber et al., 2009). Also sand content was included in the model to represent the soil texture. The variables were transformed to normal distribution according to Templeton (2011). The model was fit with maximum-likelihood estimation ('sem' function in lavaan; Rosseel, 2012). Since multivariate normality was not met in every model, we used Satorra–Bentler correction to obtain robust fitting statistics (estimator = 'MLM'). In the forest model, the path coefficient of pH to PMOR was constrained to zero since this improved model fit. In the forest models of the single regions, the path coefficient of USC-γ to PMOR was constrained to zero since USC-γ was absent in many forest soils.

3 | RESULTS

3.1 | Influence of land use, soil type, and soil texture on PMORs

Uptake of atmospheric CH_4 was detected in all 299 topsoils. PMORs varied between 0.006 and 1.695 ng CH_4 g^{-1} DW hr^{-1} and were significantly higher in forest than in grassland soils ($mean_{forest} = 0.60$ ng CH_4 g^{-1} DW hr^{-1}, $mean_{grassland} = 0.31$ ng CH_4 g^{-1} DW hr^{-1}, $p < .001$; Figure 1a). This difference between forest and grassland soils was significant in all regions ($p_{ALB} < .001$, $p_{HAI} < .001$, $p_{SCH} < .01$; Figure 1b). In the forest soils, PMORs did not vary among regions, but in grassland soils PMORs were highest in ALB, lowest in HAI, and highly variable in SCH, presumably due to the high diversity of soil types and textures in this region.

In the forest soils, PMORs did not differ with respect to soil texture (Figure S1a), but in grasslands PMORs were highest in loamy clay and loamy silt and lowest in loamy sand, silty clay, and sandy loam soils (Figure S1b). However, in the silty clay and loamy sand textures of the forest, soils' PMORs were higher than in in grassland soils of similar texture. High clay content appeared to have a generally negative effect in the ALB region (both forest and grassland sites) but a positive effect in the SCH region (grasslands only, Figure S2). Sand content therefore resulted in opposite trends in these two regions. Sand content was mostly high in SCH and typically low in the ALB region grasslands (Figure S2c,f).

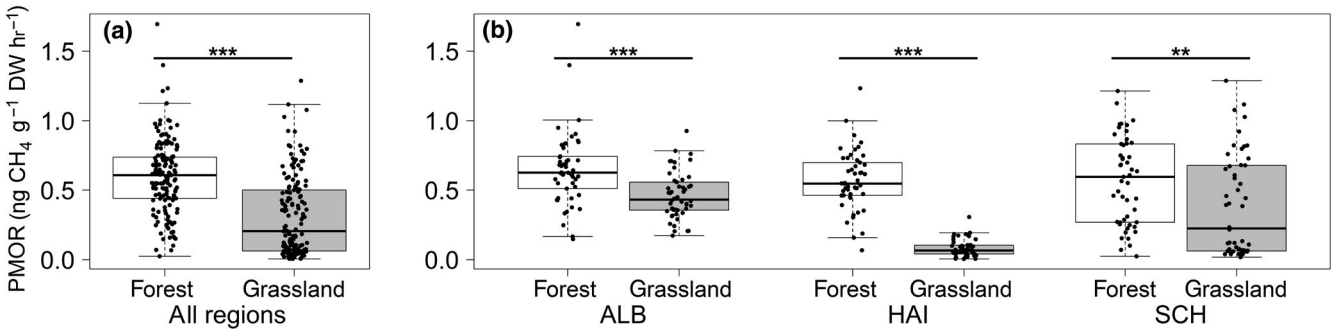

FIGURE 1 Potential methane oxidation rates (PMORs) in forests and grasslands. PMORs (a) including all regions separated into forests and grasslands and (b) in the regions Schwäbische Alb (ALB), Hainich (HAI) and Schorfheide (SCH) in forests and grasslands, significance codes: $p < .01$ (**), $p < .001$ (***), $n = 299$

3.2 | Influence of forest management, tree species, and grassland land-use intensity on PMORs

PMORs in forest soils were neither correlated to the ForMI nor to its components (proportion of non-native tree species, harvested tree biomass, and proportion of dead wood showing signs of cut, Figure S3). However, the dominant tree species did significantly affect PMORs, with lowest CH_4 oxidation in oak, and highest in beech and spruce forests (Figure 2). Oak were only in slightly loamy sand soils in SCH region. However, when only this soil texture and region were considered, PMORs were still significantly lower in oak than beech forests ($p < .05$).

In contrast to forests, where management showed no influence, the LUI in grasslands was negatively correlated with PMORs when all regions were included ($r_{LUI} = -.27$, $p < .001$; Figure S4a). When grasslands of all regions taken together were categorized into low and high LUI, PMORs were reduced by about 40% in high as compared to low LUI grassland soils (Figure 3a). With respect to the single components of LUI, fertilization decreased PMORs by about 20% (Figure 3b). Considering all grassland sites, grazing intensity and mowing frequency had no significant effect on PMORs (Figure 3c,d). Grassland management also affected the concentrations of NH_4^+ in soil, which were higher in non-fertilized compared to fertilized grasslands (Figure S5b), while NO_3^- concentrations were higher in fertilized than in non-fertilized soils (Figure S5f).

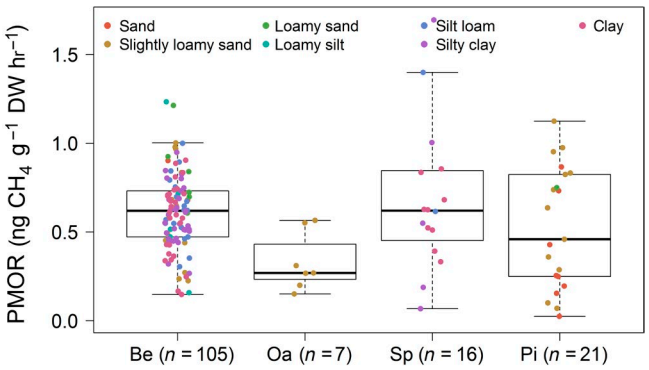

FIGURE 2 Effect of tree species (Be = beech, Oa = oak, Sp = spruce and Pi = pine) on potential methane oxidation rates (PMORs) in the forests including all regions. Coloured points indicate soil texture, $n = 149$

3.3 | Correlations of soil properties with PMORs

Considering all forest soils, PMORs were neither correlated with water holding capacity nor with organic carbon and total nitrogen content (Figure S6a–c). PMORs were, however, negatively correlated with bulk density across all forest soils ($r_{bd} = -.17$, $p < .05$; Figure S6e). In ALB and HAI, pH was negatively correlated with PMORs, while in SCH pH was positively correlated with PMORs (Figure S6d). However, pH was generally lower in SCH than in ALB or HAI (pH_{ALB}: 3.3–6.9, pH_{HAI}: 3.85–7.15, pH_{SCH}: 3.2–3.77). The thickness of the organic layer measured in the natural habitat, but which was not included in the PMOR measurements in the microcosms, had an effect on PMORs only in the HAI region, with a positive correlation between PMORs and the thickness of the organic layer ($r_{bd} = 0.34$, < 0.05, Figure S6g).

In contrast to the forest soils, PMORs in grassland soils were positively correlated with soil organic carbon and total soil nitrogen content ($r_{OC} = .60$, $r_{Ntot} = .67$, $p < .001$; Figure S7a,b). PMORs increased with increasing soil water holding capacity, but decreased with increasing bulk density ($r_{whc} = .80$, $r_{bd} = -.77$, $p < .001$; Figure S7c,e) when all grasslands were considered together, but not for the HAI region alone. Concentrations of both, NH_4^+ and NO_3^- were positively correlated with PMORs in the grasslands ($r_{NH_4} = .38$, $r_{NO_3} = .40$, $p < .001$; Figure S7g,h) and this was most pronounced in the SCH region. The effects of the mentioned soil physicochemical conditions were usually most pronounced in the SCH region.

3.4 | Influence of land use, soil type, and soil texture on MOB

In a preselection of 30 topsoil samples, no methanotrophs belonging to *Verrucomicrobia* or *Methylocella* (*Alphaproteobacteria*)

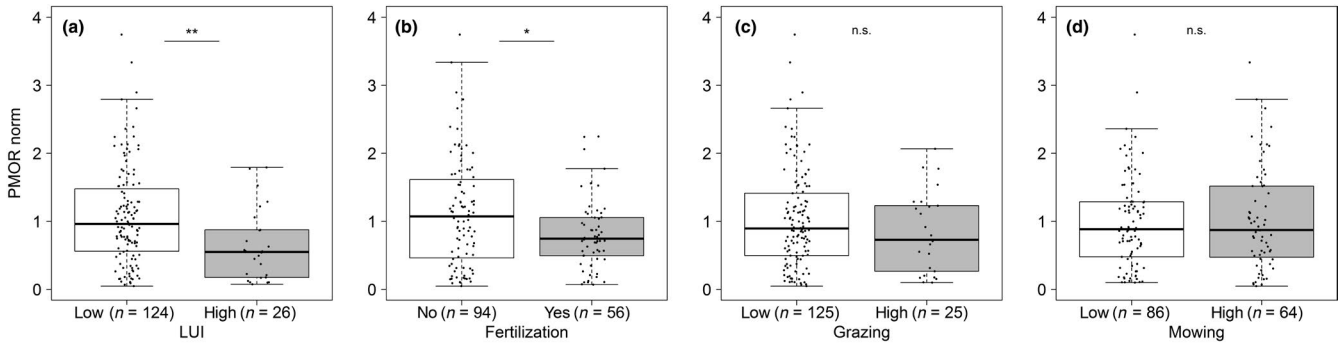

FIGURE 3 Effects of (a) land-use intensity index (LUI), (b) without (no) and with (yes) fertilization, and low and high intensity of (c) grazing and (d) mowing on normalized potential methane oxidation rates (PMORs norm) in the grassland soils. PMORs norm was calculated by dividing the PMORs of each plot by the mean PMORs of the respective region; significance codes: $p < .05$ (*), $p < .01$ (**), $n = 150$

were detected with specific PCR assays; hence, these assays were not performed for all 299 soils (data not shown). The primer pair A189f/mb661 (which targets a broad range of proteobacterial methanotrophs) yielded specific PCR products only in grassland soils from SCH while no specific products were detected in the other regions (data not shown). In contrast, we detected methanotrophs belonging to USC-α and USC-γ clades in most soils but with land-use type (forests vs. grasslands) and region-specific abundance distributions (Figure 4). USC-α abundance varied widely, from 2.8×10^4 to 8.7×10^8 *pmoA* gene copies per gram dry soil and occurred in all forest soils, but in only 56% of the grassland soils (Figure 4). USC-γ abundance ranged from 2.8×10^3 to 3.8×10^6 *pmoA* gene copies per gram dry soil and was detected in almost all grassland soils, but present only in approximately 30% of the forest soils (Figure 4). The median abundance of USC-α *pmoA* gene was almost 100 times higher in the forest than in the grassland soils in all regions ($p < .001$). In forest soils, USC-α *pmoA* gene abundance was about 50-fold higher in SCH than in either HAI or ALB. In contrast to USC-α *pmoA*, gene abundance of USC-γ was about 100 times higher in grassland than in forest soils. However, trends differed between the exploratories. In the ALB region, for example, USC-γ abundance was only twice as high in forest than in grassland sites.

3.5 | Influence of forest management, tree species, and grassland land-use intensity on MOB

USC-α gene abundance was higher in oak- and pine-dominated forests compared to spruce and beech forests while USC-γ gene abundance was higher in beech and spruce forests (Figure S8). USC-α did not correlate with ForMI, but there was a negative correlation between harvested tree biomass and USC-α (Figure S9c). USC-γ positively correlated with ForMI, non-native tree species and harvested tree biomass (Figure S9a–c). In the grasslands, there was no correlation between abundances of USC-α or USC-γ and LUI (Figure S10) and there was also no difference in USC-α and-γ copy numbers between high and low LUI or its components (Figure S11).

3.6 | Correlations of MOB abundance with soil properties and with PMORs

Upland soil cluster-α and USC-γ *pmoA* gene copy numbers responded differently to abiotic soil properties (Figures S12–S15). In forests for instance, USC-α gene copy numbers per gram soil were negatively correlated to organic carbon ($r_{Corg} = -.70$, $p < .01$; Figure S12a), whereas USC-γ abundance was positively correlated with organic carbon and nitrogen content ($r_{Corg} = .70$, $p < .001$; Figure S14a). Overall, USC-α and USC-γ abundances were differentially correlated with pH, whereas USC-α abundance was negatively correlated with pH ($r_{for} = -.70$, $r_{gras} = -.32$, $p < .001$; Figures S12d and S13d), USC-γ abundance was positively correlated with pH ($r_{for} = .54$, $r_{gras} = .39$, $p < .001$; Figures S14d and S15d).

Upland soil cluster-α abundance was negatively correlated with NH_4^+ content, whereas USC-γ abundance was positively correlated in the SCH region only ($r_{USC\alpha} = -.32$, $r_{USC\gamma} = .47$, $p < .05$; Figures S13g and S15g). USC-α and USC-γ *pmoA* gene abundances were not correlated with NO_3^- content in the grassland soils (Figures S13h and S15h).

In the forests, USC-α *pmoA* abundance correlated positively with PMORs including all regions, as well as in each of the three regions ($r_{for} = .18$, $p < .05$; $r_{ALB} = .57$, $r_{HAI} = .51$, $r_{SCH} = .68$, $p < .001$; Figure 5a). In the forests, there were no positive correlations between USC-γ *pmoA* abundance and PMORs (Figure S16a) and in the grasslands there were no positive correlations between PMORs and USC-α abundance (Figure S16b). However, in grasslands USC-γ *pmoA* copy numbers were positively correlated with PMORs when all grasslands were taken together, and also in each of the three regions (soils $r_{gra} = .44$, $r_{ALB} = .53$, $r_{HAI} = .53$, $r_{SCH} = .59$, $p < .001$; Figure 5b). When related to MOB abundance, PMOR was lower in forest than in grassland soils (Figure S17a). Within the forest soils, PMOR related to MOB was lowest in SCH region while in grasslands it was highest in SCH region (Figure S17b).

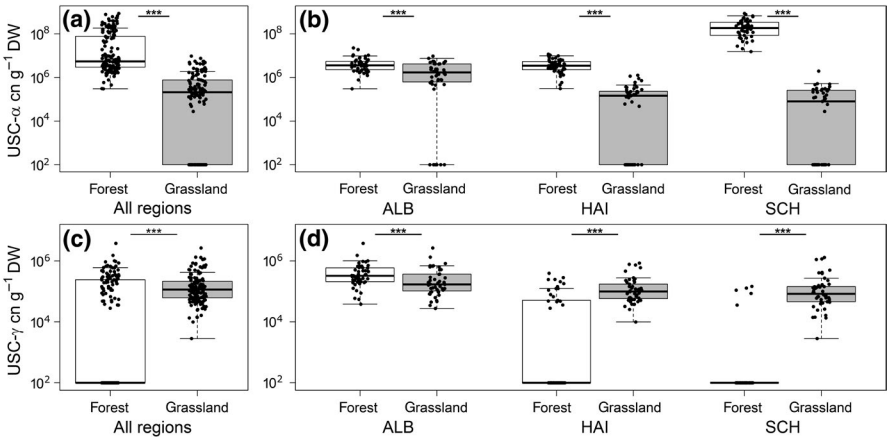

FIGURE 4 Abundance of *pmoA* copies of Upland soil cluster-α (USC-α) in (a) all regions (b) in the regions Schwäbische Alb (ALB), Hainich (HAI) and Schorfheide (SCH) in forest (white) and grassland (grey) soils. Abundance of Upland soil cluster-γ (USC-γ) in (c) all regions (d) in the regions Schwäbische Alb (ALB), Hainich (HAI) and Schorfheide (SCH). Abundances below detection limit were set to 100. $p < .001$ (***), $n = 295$

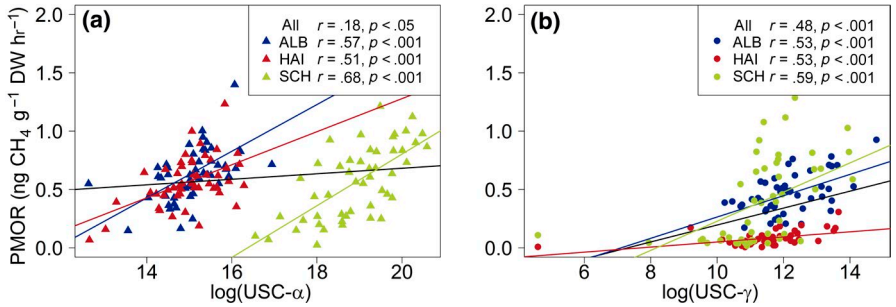

FIGURE 5 Correlation of potential methane oxidation rates (PMORs) with (a) Upland soil cluster-α (USC-α) abundance in forest soils and with (b) Upland soil cluster-γ (USC-γ) abundance in grassland soils. The colours represent the different regions Schwäbische Alb (ALB), Hainich (HAI) and Schorfheide (SCH). The significance levels reported are based on Pearson's coefficient

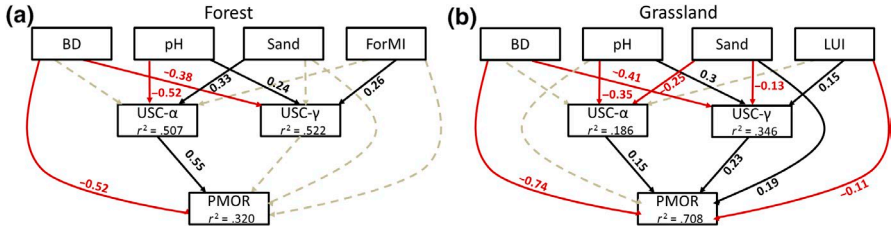

FIGURE 6 Results of structural equation modelling showing the direct and indirect effect of soil properties and land-use intensity on PMOR for (a) all forest and (b) all grassland soils. The numbers at the lines show the standardized path coefficients. Significant ($p < .05$) paths are shown as black (positive effect) or red (negative effect) lines. Non-significant ($p > .05$) paths are shown in dashed grey lines. Amount of variance explained by the model (r^2) is listed for the response variables. BD, bulk density; sand, sand content (%); ForMI, forest management index; LUI, land-use intensity index; PMOR, potential methane oxidation rate; USC-α, abundance of Upland soil cluster-α copy numbers per soil; USC-γ, abundance of Upland soil cluster-γ copy numbers per soil. $\chi^2 = 1.079; 0.255$, $df = 2;1$; $p(\chi^2) = .583; .628$, CFI = 1;1, RMSEA = 0; 0, SRMR = 0.011; 0.006 in (a) and (b) respectively

3.7 | Direct and indirect effects of soil parameters and land-use intensity on PMOR

Generally, a larger part of PMOR variance could be explained in grasslands compared to forests (Figure 6). Bulk density had strong direct negative effects on PMOR in both, forests and grasslands (Figure 6a,b). pH had no direct effects but indirect effects on PMOR via the MOB abundances, with a negative effect on USC-α abundance and a positive effect on USC-γ abundance. Sand content had an overall positive effect on PMOR in grasslands and an indirect positive effect via abundance of USC-α in forest. However, when looking at the regions separately, the soil sand content had only a positive effect on PMOR in the ALB forest region (Figure S18a). While the forest management had no effect on PMOR, land-use intensity of grasslands had a direct negative effect on PMOR but an indirect positive effect via USC-γ abundance. However, the overall effect was negative and when looking at the regions separately there was only a direct negative effect (Figure S19a,b). Only in SCH grasslands, LUI had no direct effect on PMOR. MOB abundance had a direct effect on PMOR almost all regions. In forests, USC-α abundance had a strong direct positive effect on PMOR, whereas USC-γ showed no direct effect on PMOR (Figure 6a; Figure S19a–c). In grasslands, both USC-α and USC-γ had direct positive effects on PMOR and here the effect of USC-γ was stronger than that of USC-α (Figure 6b; Figure S19a–c). Only in grasslands of the SCH region, MOB abundance had no influence on PMOR.

4 | DISCUSSION

4.1 | Potential methane oxidation rates and soil parameters

Potential atmospheric CH_4 oxidation rates (PMORs) were generally about two times higher in forest than in grassland soils. This accords with meta-analyses of CH_4 oxidation rates in different habitats (ecosystem-level measurements) that identified 2.5-fold higher CH_4 oxidation rates in forests than in other ecosystems and about two times higher in forest than in herbaceous ecosystems (Dutaur & Verchot, 2007; McDaniel et al., 2019). Compared to these studies, our PMORs were 1.5–2 times higher than the in situ CH_4 fluxes. This may be due to the fact that we analysed soil only from the layer with the highest potential for CH_4 oxidation (Kolb, 2009) and adjusted the water content to its optimal value for CH_4 oxidation (Gulledge & Schimel, 1998). Our measurements did not include deeper soil layers, which may be a source of CH_4. However, by standardizing moisture, we reduced variation found in the field, permitting better

analyses of the influence of drivers such as soil properties or MOB on PMORs. We consider the measured abundances of MOB and the standardized PMORs as proxies that integrate CH_4 uptake and MOB activity dynamics over time.

Temperature and precipitation can influence methane oxidation. For instance, Van Den Pol-van Dasselaar et al. (1998) found highest methane uptake in soils with high temperature and intermediate soil moisture content. In our study, PMORs vary between the different regions in grassland but not in forest soils. As the overall climate is rather similar between grassland and forest sites within the same region this indicates that climatic differences cannot explain much of the differences of PMOR in grasslands between the three regions. In addition, the differences in mean temperature between the regions are relatively small. In a meta study, mean annual temperature and annual rainfall had only a weak correlation with atmospheric methane oxidation (Dutaur & Verchot, 2007). So, the differences in temperature and precipitation might be too small between the regions to induce large differences in PMORs. The variation of PMOR in grasslands was likely caused by other factors, as for example differences in soil properties between the three regions. In SCH, soil texture is sandier than in the other two regions where silty, loamy, and clayey soil textures dominate. In HAI grasslands, PMORs were 4.5-fold to 5.5-fold lower than in the other grasslands. The soils of this region are generally denser, which may be a limiting factor for PMORs. The SEM indicated a general negative effect of bulk density in forests and grasslands. The high variability in SCH region might be explained by high variability in OC content of the soils.

Interestingly, many factors that correlated with PMORs in grasslands did not correlate with PMORs in forests. Within the grasslands, the PMORs increased with increasing water holding capacity but in the forest soils, water holding capacity did not have a significant effect on PMORs. Also, soil textures that were associated with low PMORs in grasslands were associated with higher PMORs in forests. These findings suggest that ecosystem type is an important driver of PMORs and that a response to soil physicochemical conditions is specific to the type of ecosystem. The high PMORs in loamy grassland soils of the SCH, however, could also be a result of their high OC concentrations. The organic layer of forest soils has been reported to reduce CH_4 oxidation in soils, probably acting as a diffusion barrier for CH_4 (Saari et al., 1998). However, we found no negative effect of the thickness of the organic layer (determined at each forest site during sampling, but the organic layer material itself was not included in the PMOR measurement) on PMORs. To the contrary, in the HAI region, the organic layer thickness had a slight positive effect on PMORs. It may be that canopy cover and the presence of an O horizon is responsible for the different responses of PMORs to soil factors in forests and grasslands. Canopy cover and O-horizon inhibit the increase in water content of the upper mineral soil layers after rainfall events (Li et al., 2014). Lower water content could, in turn, hamper gas diffusive transport in soils.

Bulk density and pH had an influence on PMORs in almost all grassland and forest soils of all three regions. PMOR generally decreased with increasing bulk density. Higher bulk density indicates low soil porosity and pronounced soil compaction which, considered together, may result in lower diffusion capacity of atmospheric gases into the soils. This, in turn, could lead to lower CH_4 availability in the soil and thus lower the CH_4 oxidation rates (Malghani et al., 2016). It is worth noting that the original bulk density in the field had an effect on CH_4 oxidation even after sieving and re-compaction of the soil, indicating a legacy effect of the former natural conditions. Also Sitaula et al. (2000) reported that soil compaction led to decreased CH_4 uptake even after compaction was removed by sieving of the soil samples. The response of PMORs to pH differed between forests and grasslands. While in forest soils, PMORs had an optimum at around pH 4, in grasslands PMORs increased with increasing pH in two out of three regions. Soils have been shown CH_4 oxidation over a wide range of pH values and incubation of forest soils demonstrated CH_4 oxidation from pH 3–7.5 even though the optimal pH for CH_4 oxidation ranged from 4 to 7.5 (Amaral et al., 1998; Benstead & King, 2001; Saari et al., 2004). Sitaula et al. (1995) observed an increase in CH_4 oxidation when soil from a pine forest was irrigated with acidic water. In contrast, CH_4 oxidation has been reported to decrease with lower pH in grasslands (Hütsch et al., 1994), which is in accordance with our results. Also in arable soils, strong inhibition of CH_4 oxidation was reported when the soil pH was lowered from 8 to 7.1 (Hütsch, 2001). Thus, our findings underline that pH has a substantial impact on CH_4 oxidation; however, its influence differs between different ecosystem types. While in forests CH_4 oxidation is favoured by slightly acidic conditions, in grasslands CH_4 oxidation is higher in neutral soils. We found that the effect of pH was direct only in forest sites in the ALB region, while in the other cases the observed effects of pH were indirect via the abundances of the two types of methane-oxidizing bacteria. This indicates that there are different MOB communities with different pH optima in forests and grasslands.

We note that the variation in PMORs was far greater within the grasslands than in the forests and was region-dependent within the grasslands. PMORs were generally higher in forest than in grassland soils, indicating that forest soils act as robust sinks for CH_4 over a wide range of different physicochemical soil conditions.

4.2 | Drivers of MOB abundances and relationship with PMOR

We measured MOB abundances in nearly 299 different soils, thus yielding a comprehensive dataset to connect MOB with soil physicochemical soil properties and PMORs. The composition and importance of the MOBs seem to be ecosystem type- and region-specific. USC-α *pmoA* abundances were positively correlated with PMORs in forests of all regions, but USC-α was absent in many grasslands. In contrast, USC-γ *pmoA* were consistently present in the grasslands and were positively correlated with PMORs in all of the grasslands but in none of the forest regions. This indicates the far greater importance of USC-α MOBs for CH_4 oxidation in forest soils and that of USC-γ MOBs for CH_4 oxidation in grasslands. In some grasslands,

USC-α abundance might be an additional driver of CH_4 oxidation, even though it has a smaller effect on CH_4 oxidation than USC-γ abundance. USC-α MOBs have been previously detected in forest soils and 16S rRNA gene amplicon datasets demonstrate that they occur in forest soils (Tveit et al., 2019). A recent study found that USC-γ was dominant in upland grassland soils from a region in China (Deng et al., 2019). In combination with their wide occurrence also in our samples provides evidence that USC-γ is an important MOB in grassland soils in different regions of the world.

Soil pH was the most important predictor of USC-α and -γ gene abundances, with USC-α preferring more acidic and USC-γ preferring neutral soils. The lower pH of the forests and more neutral pH in the grasslands may therefore explain, in part, the distribution patterns of the two USC groups. However, USC-α were also present in neutral soils. This confirms results of former studies (Kolb, 2009; Kolb et al., 2003). However, the negative correlation of USC-α abundance and pH is also surprising, given the latest findings on the physiology of atmospheric MOB belonging to the USC-α *M. gorgona*. The optimal pH for growth of *M. gorgona* is at an almost neutral pH of 6.5–7, but other *Methylocapsa* strains that were able to grow at atmospheric CH_4 concentrations had a lower pH optimum of 5–6.2 (Tveit et al., 2019).

PMOR per unit biomass was generally lower in forest than in grassland soils. This might be due to the different microbial communities in these land-use type which might have a different specific activity. In forests, PMOR per unit biomass was lowest in SCH region that was also the region with the highest bulk density among forest soils. The PMOR per unit biomass might thus be influenced by gas diffusive transport which is lower in soils with high bulk density and thus a higher abundance of MOBs might be needed to oxidize similar amounts of CH_4.

4.3 | Effects of grassland land-use intensity and forest management

We found that grassland land-use intensity had a negative effect on PMORs, which supports our initial hypothesis. Structural equation modelling showed a direct negative effect of LUI across all regions. Only in SCH region, no effect on PMOR was detectable. This region is less intensively managed in terms of fertilization compared to the other two regions. This may explain why there was no effect of LUI on PMORs in SCH region since fertilization in particular negatively influenced PMORs, with 20% lower rates in fertilized compared to non-fertilized soils. Ammonium ions, that are a component of fertilizers, are known to inhibit methane monooxygenase (Schnell & King, 1994). However, we could not detect higher ammonium concentrations in the fertilized soils. Since we do not know the exact date of fertilization, and as the ammonium concentration in soils is highly dynamic, the concentration at our sampling date may not have reflected the mean ammonium concentrations over the year. There may also be a legacy effect of formerly high ammonium concentrations from fertilization that negatively influences the MOB community over the long term. Interestingly, fertilization had no effect on the abundances of MOBs but it did have an effect on PMORs. With respect to the other two components of grassland land-use intensity, we could not detect any significant effect of either grazing or mowing on PMORs. However, a high LUI, which integrates fertilization, grazing, and mowing, reduced PMORs by 40% in comparison to grasslands with low land-use intensity. Hence, the latter two factors did have an additive negative effect on PMORs. The reduction of PMORs by grazing and mowing may have been due to soil compaction as caused to animal trampling and mowing machines. However, only in combination with N-fertilization did soil compaction lead to a reduction in CH_4 oxidation in these soils. Heavy grazing reduces water infiltration into soil (Abdel-Magid et al., 1987) and thus also alters gas diffusive transport into soil.

Our study investigated PMORs over many different grasslands and land-use intensities and we can confirm that fertilization has a negative effect on PMORs over different soil types over a regional gradient of more than 800 km, in contrast to previous studies reporting somewhat contradictory effects of fertilization on CH_4 oxidation (Imer et al., 2013; Liu & Greaver, 2009). We thus conclude that by a reduction of land-use intensity, especially N-fertilization, the CH_4 sink function of temperate grasslands could be improved or the other way around, an intensification of grassland land use bears the risk of the reduction of methane uptake in grassland soils.

Within the investigated 149 forest soils, we did not observe any effect of forest management on PMORs. This suggests that the ability of temperate forest soils to serve as CH_4 sinks is not substantially affected by commonly applied forest management practices. Homogenization of the soils prior to measuring PMOR may have partly removed negative effects that were consequences of forest management practices, such as soil compaction due to forest machinery. However, we still see a legacy effect of the natural bulk density. Hence, it is unlikely that forest management effects were completely eliminated by the treatment of the soil before PMOR measurements. It is likely that inhibition of PMORs is most prevalent in the logging trails, which were excluded from soils sampling in our study. A closer sampling of the forest soils may be necessary to better understand the influence of management in forests. However, based on our data, we must reject our initial hypothesis of a negative effect of forest management on PMORs.

We found that the dominant tree species had some effect on PMORs. Even though the literature indicates that spruce forest soils exhibit a lower capacity to oxidize CH_4 than beech forest soils (Borken & Beese, 2006; Degelmann et al., 2009), we could not detect significant differences between beech-dominated and coniferous forests (pine or spruce) across all forest sites. However, PMORs were lower in oak than in beech dominated forests. In oak-dominated forests, USC-α abundance and soil respiration rates were also reduced, indicating the presence of inhibitory substances in the soil that hamper microbial activity. Bárcena et al. (2014) also found that CH_4 oxidation rates were higher in spruce than in young oak forests. However, others have reported that oak forests have higher

rates than spruce and pine forests (Reay et al., 2005). It is likely that tree species alone is not the most important factor impairing PMORs. Soil physicochemical conditions can differentially influence CH_4 oxidation with respect to different tree species. For example, while higher water content increased CH_4 oxidation in spruce soils, it decreased CH_4 oxidation in scots pine and larch soils (Menyailo & Hungate, 2003). Possibly, there are optimal soil types and textures for a certain tree species and thus, a specific main tree species could maximize CH_4 oxidation in a particular soil.

Our results clearly demonstrate that forest soils are an important sink for atmospheric CH_4 and that this is largely stable over different physicochemical conditions and forest management practices. Since PMORs were higher in forests than in grasslands, afforestation has the potential to enlarge the global CH_4 sink of soils and thus, help mitigate global warming by decreasing atmospheric CH_4 concentrations.

5 | CONCLUSIONS

PMORs are differentially controlled in forest and grassland soils. Our survey demonstrates that forests are an important and robust sink for CH_4 over a wide range of different physicochemical soil conditions while in grasslands PMORs are clearly more influenced by site-specific soil properties. Additionally, we detected a negative effect of grassland land-use intensity, especially fertilization, while the different forest management practices did not affect PMORs. Thus, reduction in grassland management intensity as well as afforestation may increase the capacity of soils to serve as CH_4 sinks.

Furthermore, our results strongly suggest that USC-α and USC-γ have land-use type specific distributions, with USC-α the dominant group in forests and USC-γ the dominant group in grasslands. Also, the direct positive correlations between PMORs and USC-α in forests and between PMORs and mainly USC-γ in grasslands indicate that USC-α is the major microbial group responsible for the CH_4 sink capacity in forests and USC-γ is the major group responsible for the CH_4 sink capacity of grasslands. Finally, the study also revealed that different sets of site parameters control the microbial methane capacity sink in forests and grasslands.

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DATA AVAILABILITY STATEMENT

Data are stored in BExIS and available on request according to the rules of BExIS (https://www.bexis.uni-jena.de/).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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