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Microbial mechanisms for methane source-to-sink transition after wetland conversion to cropland

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ABSTRACT

Wetland conversion to cropland substantially reduces methane (CH_4) emission, turning a source into a sink on many occasions; how various microbial processes contribute to this source-to-sink transition remains elusive. We addressed this issue by examining the net CH_4 flux, CH_4 production potential, CH_4 oxidation potential, and functional genes associated with methanogenesis and methanotrophy in a pristine wetland and a 23-year cultivated cropland in the Sanjiang Plain, China. The study confirmed that wetland conversion to cropland turned a CH_4 source of 44.93 ± 10.17 g $CH_4 \cdot m^{-2} \cdot yr^{-1}$ to a small CH_4 sink of −0.056 ± 0.051 g CH_4 $m^{-2} \cdot yr^{-1}$. The proportion of total CH_4-related genes, methanogenesis genes, as well as the CH_4 production marker genes – *mcr* were significantly decreased by 24.14 %, 32.10 %, and 97.89 %, respectively in cropland. The proportions of methanotrophic marker genes, *pMMO,* and the sum of *sMMO* and *pMMO* were significantly increased by 48.74 % and 22.79 % after wetland cultivation. The 23-year cultivation yielded suppressing impacts on methanogenesis and *mcr* genes throughout the four seasons while stimulating effects on the functional genes of *sMMO*, *pMMO,* and *MMO* in spring and summer. The proportions of CH_4-related genes decreased along soil depth in wetland and cropland, while *pMMO* and *MMO* slightly increased in the depth of 20–60 cm in cropland. A global synthesis supported this microbial mechanism for the CH_4 source-to-sink transition, indicating the strong methanogenesis suppression and slight methanotrophy enhancement in explaining the source-to-sink transition after wetland conversion to cropland. This mechanism should be incorporated into CH_4 models to predict CH_4 dynamics under land-use change.

1. Introduction

Methane (CH_4) is a potent greenhouse gas that has 34 times the global warming potential of carbon dioxide (CO_2), and its atmospheric concentration has increased 2.5 times since the Industrial Revolution (Forster et al., 2007; IPCC, 2018; IPCC, 2021). However, there are still large uncertainties in predicting terrestrial CH_4 flux under the changing environment at various temporal and spatial scales (Bridgham et al., 2013; Feng et al., 2020; Nisbet et al., 2014; Yvon-Durocher et al., 2014) due to our incomplete understanding of biological processes and abiotic controls of CH_4 cycling (Bansal et al., 2018), as well as its spatial heterogeneity.

The surface CH_4 flux is the net balance of two counteracting microbial processes, CH_4 production (methanogenesis) and CH_4 oxidation (methanotrophy) (Chen et al., 2013; Liu and Whitman, 2008; Segers, 1998). Methanogenesis is carried out by methanogens from the domain archaea. Under anoxic conditions, soil methanogens produce CH_4 by splitting fermentation products such as acetate, reducing CO_2 with hydrogen (Demirel and Scherer, 2008), or using a variety of methylated compounds under hypersaline conditions (Serrano-Silva et al., 2014).

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The methyl coenzyme-M reductase (*mcr*), which is responsible for reducing the methyl group bound to coenzyme M in the last step of all methanogenic pathways, is typically used as a functional gene marker (Luton et al., 2002). Methanotrophy is carried out by methanotrophs, primarily bacteria and archaea (Brindha and Vasudevan, 2017; Du et al., 2021). The first step of the biochemical pathway for CH_4 oxidation is employed by CH_4 oxidation bacteria; it involves soluble and/or particulate methane monooxygenase enzymes (*sMMO* and *pMMO*, respectively) (Hakemian and Rosenzweig, 2007). *pMMO* is more prevalent as all methanotrophic microorganisms produce it in both wetland and upland environments, while *sMMO* could only express by only one genus, primarily in wet, copper-deficient environments (Hanson and Hanson, 1996; Hakemian and Rosenzweig, 2007). Thus, *pMMO* may play a more critical role in the consumption of atmospheric CH_4 than *sMMO*. Therefore, investigating synchronous changes of methanogens and methanotrophs with CH_4 dynamics could provide insights into the microbial mechanisms for the CH_4 dynamics (Hazard et al., 2021; Robert Burger et al., 2021).

As the largest natural source of CH_4, wetlands emit approximately 138–165 Tg CH_4 yr^{-1}, which contributes 20–25 % of the total global CH_4 budget (Bridgham et al., 2013; Jackson et al., 2020; Lyu et al., 2018; Rosentreter et al., 2021). Wetland conversion is the predominant disturbance leading to wetland loss across the globe (He and Zhang, 2001; Stein, 2020; Tate, 2015; Zou et al., 2018), which dramatically reduces CH_4 emissions (Nisbet et al., 2014; Saunois et al., 2020) at the expense of CO_2 emissions enhancement and soil carbon loss (Sheng et al., 2021). For example, a *meta*-analysis confirmed that land use and land cover changes in riparian wetlands significantly decreased the CH_4 emission across the globe (Tan et al., 2020); wetland loss had accumulatively reduced 28.0–54.1 Tg C CH_4 emission in Chinese wetlands during 1949–2009, and 62 % was from northeastern China (Xu and Tian, 2012); another study reported an accumulative 36 (24–57) Tg C reduction in CH_4 emission in northeast China from 1960 to 2009 and ~86 % of the reduction was attributed to the extensive marshland conversion to cropland (Li et al., 2012). Eventually, wetland conversion to cropland substantially altered soil physicochemical properties (Li et al., 2014; Song et al., 2012; Xie et al., 2017), redox properties (Liu et al., 2020), and soil microbial communities (Hou et al., 2018; Meyer et al., 2020) and further caused a decline in CH_4 emission (Li et al., 2012). Yet it remains unclear how methanogens and methanotrophs play a role in this transition.

The relative abundance of methanogenesis and methanotrophs vary along the soil profile and across seasons, leading to the fluctuation of CH_4 emission across seasons and along the soil profiles. Dramatic variations in CH_4 flux have been widely observed over seasons, which are regulated by soil moisture and temperature (Feng et al., 2020; Martinez-Cruz et al., 2015). A *meta*-analysis showed that CH_4 emission and CH_4 production increased markedly with seasonal increases in temperature, which were derived from methanogens (Yvon-Durocher et al., 2014). A study explored the methanogens and methanotrophs across seasons and found more resistance of methanogens to dramatic seasonal environment changes than that of methanotrophs (Gontijo et al., 2020). In contrast, a large variation in CH_4 production and consumption has been observed along soil profiles, speculating that this might be due to limited available organic substrates to methanogens (Jerman et al., 2017). Therefore, the large uncertainty of microbial mechanisms in the CH_4 process across seasons and along soil profiles impedes a better understanding of the CH_4 flux.

Thus, we aimed to understand how methanogens and methanotrophs regulated the changes in CH_4 emission after wetland conversion to cropland. We hypothesized that both methanogenesis suppression and methanotrophy enhancement led to a decline in CH_4 emission. To achieve this, we quantified the net CH_4 flux, CH_4 production and oxidation potentials, genes for methanogenesis, and methanotrophy along 0–100 cm soil profile in four seasons to develop a complete picture of the CH_4 cycling in response to wetland conversion to cropland.

2. Material and methods

2.1. Site description and sampling

This study was conducted at the Sanjiang Mire Wetland Experimental Station of the Chinese Academy of Sciences, located in Sanjiang Plain (133°31′E, 47°35′N) in Tongjiang City, northeastern China. The study site is characterized by a temperate humid, and subhumid continental monsoon climate. The mean annual temperature is ~2.5°, with the lowest −20° in January and the highest at 22° in July. (Li et al., 2012; Song et al., 2012). Mean annual precipitation ranges from 500 to 650 mm, with 80 % occurring from May to September (Fu et al., 2020). Two adjacent ecosystems, wetland (133 °21′ 57.87′' E, 47 °29′ 43.58′'N) and cropland (133°21′ 51.92′'E, 47 °29′ 44.25′'N) (~100 m apart), were selected as sampling sites. The wetland in our study site is dominated by *Carex meyeriana*, *Carex lasiocarpa*, and *Deyeuxia angustifolia* communities, and a 30–35 cm deep water layer covers the surface of the wetland. The cropland has been converted from the previously drained wetland since 1996 and was cultivated with soybean (*Glycine* max [L.] Merr.) during May - October every year without any fertilization (Zhu et al., 2021). The soil texture in wetland and cropland is silty loam (Kyebogola et al., 2020), with meadow soils at 0–60 cm depth and white territory soils at 60–100 cm.

We chose three sampling sites in every land use type. In each sampling site, three soil cores were excavated in October 2019 (Autumn), January 2020 (Winter), May 2020 (Spring), and July 2020 (Summer), respectively, to represent four seasons. The cylindrical, steel corer (10 cm inner diameter) was used, and the cores were dug out to collect soil. Every soil core was 100 cm in-depth, and we divided the soil cores into ten sections (every 10 cm). Three soil cores in each sampling site were mixed in layers. Therefore, we collected 240 soil samples from four seasons. All the visible roots, stones, and residues were removed. The soils were packed in polyethylene bags immediately after collection, cooled with ice packs and brought back to the laboratory in 24 h for further analysis.

2.2. Methane flux, the potentials of CH_4 production and oxidation, and soil environmental factors

The net CH_4 flux was measured at the same sites using static gas chambers every week during 2003 – 2005 (Hao, 2005; Liu et al., 2011); our recent publication showed minor inter-annual variations in the net CH_4 flux in the past ten years in those sites (Song et al., 2009; Sun et al., 2018; Sun et al., 2013; Zhu et al., 2014). The observed differences in CH_4 flux between the sites prompted the research team to investigate potential differences in soil properties and microbial communities The CH_4 production potential was measured with an incubation experiment. Specifically, a fresh soil of ~ 20 g dry weight was sealed in a bottle, and three replicates were kept. To remain in anaerobic conditions, the bottles were added with 100 ml ddH_2O and put in a 30 ℃ incubator for 5 h. Nitrogen gas was used to fill the space of the bottles after the soils were sealed to guarantee anoxic condition. When the CH_4 oxidation potential was measured, the bottles were kept open for 3 min before being sealed. The soils were kept at natural water condition and 80 % water holding capacity, and the CH_4 oxidation potential was the average of those two conditions (Wang et al., 2006). Before and after the incubations, gas was extracted, and the CH_4 concentration was measured by gas chromatography; the changes in CH_4 concentration was normalized to dry-weight soil to represent CH_4 production potential (*mpp*) or CH_4 oxidation potential (*mop*) with an equation as below:$mxp = \frac{(C-C_0)(V-100)\times 273\times 16}{1000\times 22.4\times(273+t)\times m\times t}$, where *mxp* is *mpp* or *mop*; C is the CH_4 concentration at the end of incubation; C_0 is the CH_4 concentration at the beginning of incubation; V is the volume of containers; m is the dry soil weight in each bottle and t' is the time of incubation in hours; t is temperature, and $273 + t$ is the thermodynamic temperature; 22.4 is the molar

volume of the ideal gas; 16 is the molecular mass of CH_4 (Li et al., 2021; Wu et al., 2019).

Soil water content and total carbon (TC) were measured for each soil sample. Soil water content was determined by the drying method; the difference between fresh soil and the soil dried to constant weight. TC was measured by dry combustion (about 950℃) using Multi-N/C 2100 TOC analyzer (Analytikjena, Germany). The soil temperature was obtained from the long-term automatic weather stations in adjacent wetlands and cropland.

2.3. Soil DNA extraction and sequencing

DNA was extracted from every soil sample using fastDNA® Spin Kit (MP Biomedicals, Inc., CA, USA) according to the manufacturer's instructions. The concentration and purity of extracted DNA were determined with TBS-380 (Turner BioSystems, Inc., CA, USA) and NanoDrop2000 (Thermo Fisher Scientific, Inc. MA, US), respectively. After being detected by 1 % agarose gel electrophoresis, DNA was fragmented to an average size of ~ 400 bp using Covaris M220 (Gene Company Limited, China) for paired-end library construction using NEXTflex™ Rapid DNA-Seq (Bioo Scientific, Austin, TX, USA). Adapters containing the full complement of sequencing primer hybridization sites were ligated to the blunt-end of fragments. Paired-end sequencing was performed on Illumina NovaSeq 6000 (Illumina Inc., San Diego, CA, USA) at Majorbio Bio-Pharm Technology Co., ltd. (Shanghai, China) using NovaSeq Reagent Kits according to the manufacturer's instructions (https://www.illumina.com).

2.4. Bioinformatics and statistics

To achieve high-quality reads, raw reads were used to generate clean reads by removing adaptor sequences, trimming and removing low-quality reads (reads with N bases, a minimum length threshold of 50 bp, and a minimum quality threshold of 20) using the fastp (https://github.com/OpenGene/fastp, version 0.20.0) (Chen et al., 2018). These high-quality reads were then assembled to contigs using MEGAHIT (https://github.com/voutcn/megahit, version 1.1.2) (Li et al., 2015). Contigs with the length being or over 300 bp were selected as the final assembling result. To identify the functional genes, open reading frames (ORFs) in contigs were identified using MetaGene (http://metagene.cb.k.u-tokyo.ac.jp/) (Noguchi et al., 2006). The predicted ORFs with lengths over 100 bp were retrieved and translated into amino acid sequences using the NCBI translation table (https://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html/index.cgi?chapter = tgencodes#SG1). A non-redundant gene catalog was constructed using CD-HIT (https://www.bioinformatics.org/cd-hit/, version 4.6.1) (Fu et al., 2012) with 90 % sequence identity and 90 % coverage. Then high-quality reads were mapped to the non-redundant gene catalog with 95 % identity using SOAPaligner (http://soap.genomics.org.cn/, version 2.21) (Li et al., 2008), and then we evaluated gene abundance in each sample. The genes annotations were conducted using Diamond (http://www.diamondsearch.org/index.php, version 0.8.35) (Buchfink et al., 2015) against the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/keeg/, version 94.2) with an e-value cutoff of $1e^{-5}$.

We totally obtained 150,888,735 reads annotated to the CH_4 metabolism pathway (pathway id: ko00680). Modules and enzymes table were rarefied to 1 million observations per sample, that is parts per million (PPM): $PPM_i = R_i{*}10^6/\sum_1^n(R_i)$. In which R_i represents the reads number in the ith sample; n is the total sample size. The functional genes annotated to CH_4 production, and associated enzymes (Table S1) were selected to represent methanogenesis. CH_4 consumption, including CH_4 to formaldehyde, and three formaldehyde assimilation pathways were selected to estimate methanotrophy (Table S1). Total CH_4-related genes were the sum of methanogenesis and methanotrophy. In addition, two gene markers, *mcr*, coded methyl coenzyme M (Methyl-CoM) reductase, and *MMO*, which included two types of CH_4 monooxygenase gene (*pMMO* and *sMMO*), were evaluated. The proportion of gene reads was rarefied to 1 million observations per sample. Kruskal-Wallis was used to compare the differences in those genes between wetland and cropland. The difference of gene variation across seasons and along soil profiles was estimated using ANOVA comparisons, and log transformation was used to achieve a normality (Warton and Hui, 2011). The variation was calculated as the relative changes of each gene in cropland vs wetland to represent the cultivation effects in different seasons and layers (Wang et al., 2020). The equation was: $Variation_i = (cropland_i - wetland_i)/wetland_i$, where i refer to the genes we focused on, including total CH_4-related genes, genes of methanogenesis, methanotroph, *mcr, sMMO, pMMO and MMO*.

2.5. Meta data source and processing

An extensive literature survey was conducted through the "Web of Science" database until December 5, 2021, with no restriction on the publication year. The key words are "(Wetland* Or Swamp* Or Marsh*) AND (Reclamation Or Culti* Or Land-use-change* Or conversion* Or transition*) AND (methane* Or CH_4* Or methano* Or mcrA Or pmoA)". A total of 876 papers matched the keywords. The criteria for selecting proper studies were: 1) both pristine wetland and converted farmland were included in the same study area within the same measurement time; 2) at least one of the variables related to net CH_4 flux, the abundance of methanogenesis (or/and *mcrA* genes) or methanotrophy (or/and *pmoA* genes) were reported. In total, 27 articles were selected and used for the *meta*-analysis in this study. A total of 414 pairs of observed variables of CH_4 emissions were taken from 23 publications. 8 pairs of methanogenesis variables including abundance of methanogens and copy number of *mcrA*, and 4 pairs of methanotrophy variables including abundance of methanotrophs and copy numbers of *pmoA* genes were taken from 4 publications (Fig. 5c, details in Table S3).

The differences in net CH_4 flux and microbial metrics between the pristine and the converted land-use types were evaluated with Kruskal-Wallis test. The analyses were carried out on the log-transformed data. Then, the response ratio (RR) was used to evaluate the responses of net CH_4 flux and microbes to land-use changes. RR is defined as the natural logarithm of the ratio of variables in land-use change group to that in the pristine group. To avoid the invalidation of negative value, all variables plus 10 before Log transformation. The statistical analyses and graph production were carried out by using 'ggmap', 'metafor' packages in R (version 4.0.2 on the Windows 10).

3. Results

3.1. Methane flux and potentials of CH_4 production and oxidation in wetland vs Cropland

Wetland conversion to cropland had strong impacts on net CH_4 flux, turning a CH_4 source to a CH_4 sink. Specifically, the wetland acted as a CH_4 source with a flux rate of 44.93 ± 10.17 g·CH_4 m^{-2}·yr^{-1}, while the cropland was a CH_4 sink with an annual budget of -0.056 ± 0.051 g CH_4 m^{-2}·yr^{-1} (Fig. 1a). The 23-year cultivation caused a net decline of CH_4 emission by ~ 45 ± 12 g CH_4 m^{-2} yr^{-1} in the Sanjiang Plain, northeastern China.

The potentials of CH_4 production and oxidation in soils are distinct between the wetland and cropland along soil profiles. Compared with the wetland, the potential of CH_4 production in the cropland significantly decreased from 4.14 to 1.64 mg·Kg^{-1}·h^{-1}($P < 0.05$, Fig. 1b). Across four seasons and ten soil layers (Fig. S1), the CH_4 production potential in cropland was significantly lower than wetland for 0 – 20 cm soils in autumn, 0 – 10 cm and 60 – 70 cm soils in winter, 0 – 30 cm and 60 – 70 cm soils in spring, 0 – 20 cm, 60 – 70 cm and 90 – 100 cm soils in summer ($P < 0.05$, Fig. S1). However, the cropland had higher CH_4

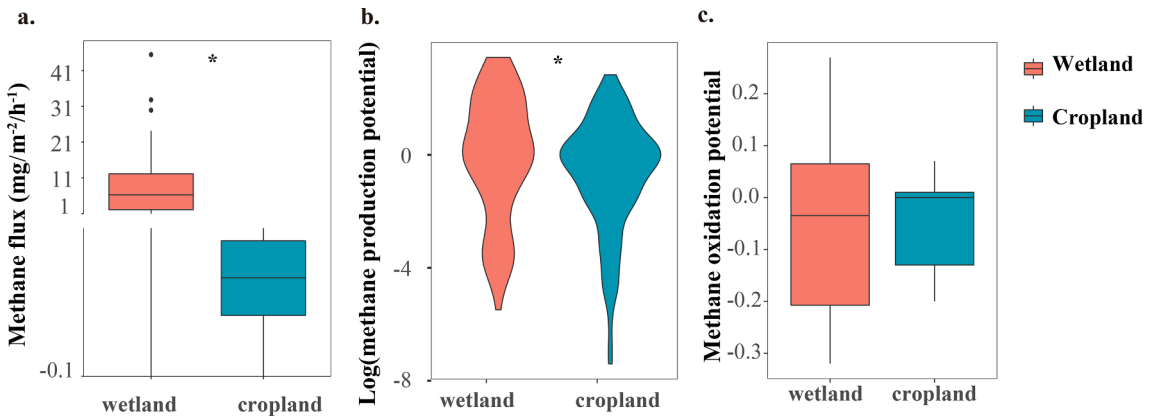

Fig. 1. Measured CH_4 processes in wetlands and cultivated cropland. a. net CH_4 flux; b. CH_4 production potential; c. CH_4 oxidation potential. "*" indicates that the differences between wetland and cropland are significant at the level of $P = 0.05$.

production potential than the wetland in 20 – 30 cm and 40 – 50 cm soils in winter, 40 – 50 cm and 90 – 100 cm soils in spring, and 20 – 30 cm soils in summer (Fig. S1). The potential of CH_4 oxidation in soils in cropland was higher than that in wetland, but not significantly (Fig. 1c).

3.2. Methane-related genes in soils from wetland vs Cropland

Cultivation exhibited significant impacts on genes for CH_4 production and oxidation processes, but the impacts were in different directions and magnitudes. Both the proportions of methanogenesis genes and methanotroph genes were significantly decreased after 23-year cultivation ($P < 0.05$, Fig. 2a, b, d), while the suppression impact on methanogenesis genes (decreased 32.10 %) was larger than that of methanotroph genes (decreased 6.98 %) (Fig. S3 and Fig. S4). Moreover, the proportion of *mcr* genes, which is the marker genes of methanogenesis, coding Methyl-CoM reductase, was significantly reduced by 97.89 % after the 23-year cultivation ($P < 0.05$, Fig. 2c). On the contrary, the proportion of *sMMO* in soils were not significantly different between wetland and cropland ($P = 0.16$, Fig. 2e), while *pMMO* in soils and the sum of these two were significantly increased by 48.74 % and 22.79 %, respectively, after wetland conversion to cropland (Fig. 2f).

3.3. Cultivation impacts on seasonal patterns of CH_4-related genes

The CH_4-related genes substantially varied across seasons, with obvious cultivation impacts on those genes. The proportions of total CH_4-related genes, methanogenesis, and *mcr* genes in the wetland substantially fluctuated across seasons, with the highest in spring and the lowest in autumn ($P < 0.001$, Table 1, Fig. 3a, b, c). At the same time, the proportion of *sMMO*, *pMMO* and *MMO* genes varied across seasons in the cropland, with the highest in summer and the lowest in winter ($P < 0.05$, Table 1, Fig. 3e, f, g), but they did not show substantial variation in the wetland (Table 1). Moreover, the cultivation impacts on CH_4-related genes varied across four seasons. Compared with those in wetland soils, the proportion of total CH_4-related genes in cropland soils was significantly lower by 15.41 %, 32.19 %, and 29.31 % in winter, spring, and summer, respectively ($P < 0.05$, Fig. 3a, Fig. S3a), and the proportion of methanogenesis genes in cropland soils were significantly lower in all seasons ($P < 0.05$), ranging from 5.04 % in autumn to 40.57 % in spring (Fig. 3b, Fig. S3b). Compared with the wetland soils, cropland soils had significantly lower values in the proportion of *mcr* genes in all seasons ($P < 0.05$, Fig. 3c), and the variations ranged from 81.45 % in autumn to 94.68 % in winter, 99.38 % in spring, and 99.74 % in autumn (Fig. S3c).

The cultivation impacts on methanotrophs and their marker genes varied across seasons. The proportion of methanotroph genes in cropland soils was slightly but significantly lower by 10.92 % than those in

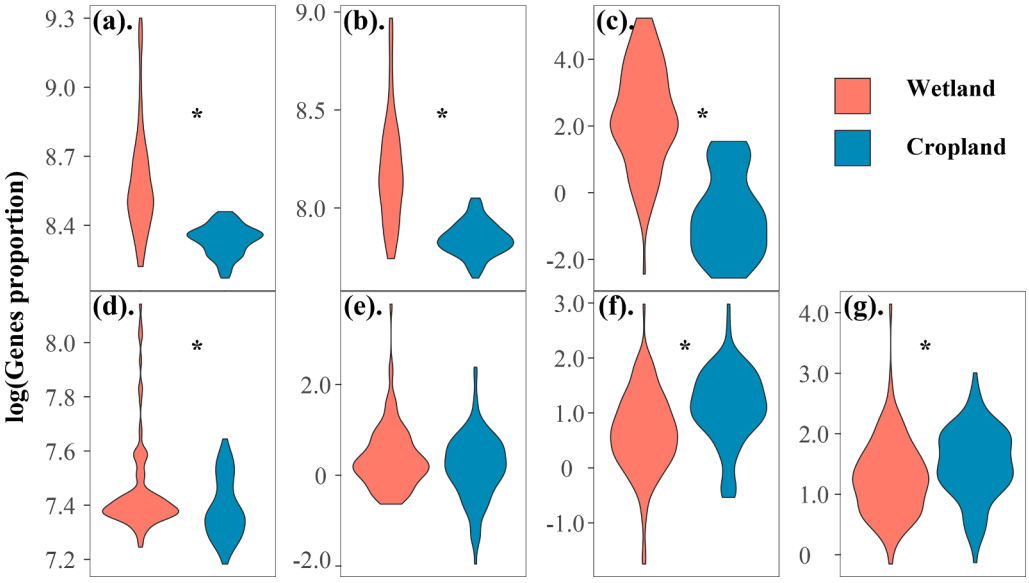

Fig. 2. Methane-related genes in soils between wetland and cropland. (a). the proportion of total CH_4-related genes; (b). the proportion of methanogenesis genes; (c). the proportion of *mcr* genes (d). the proportion of CH_4 oxidation genes; (e). the proportion of *sMMO* (encoding soluble CH_4 monooxygenase) genes; (f). the proportion of *pMMO* (encoding particulate CH_4 monooxygenase) genes; (g). *MMO* means the sum of *pMMO* and *sMMO*. "*" indicates that the differences between wetland and cropland are significant at the level of $P = 0.05$.

Table 1
Statistical test of methane production potential and CH_4-related functional genes across seasons and depth by Kruskal-Wallis.

	Wetland				Cropland			
	Seasons		**Layer**		**Seasons**		**Layer**	
	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*
Total related genes	62.37	**<0.0001**	21.45	**0.011**	6.92	0.075	16.90	0.050
Methanogenesis	63.31	**<0.0001**	20.33	**0.016**	0.61	0.90	11.87	0.22
mcr	60.39	**<0.0001**	28.11	**0.00091**	12.97	**0.0047**	11.75	0.23
Methanotroph	4.83	0.18	33.27	**0.00012**	5.13	0.16	58.79	**<0.0001**
sMMO	2.66	0.45	28.36	**<0.0001**	11.64	**0.0087**	22.62	**0.0071**
pMMO	7.68	0.053	40.23	**<0.0001**	16.80	**0.00078**	27.40	**0.0012**
MMO	3.97	0.26	38.61	**<0.0001**	15.65	**0.0013**	29.69	**0.00050**
Methane production potential	67.55	**<0.0001**	31.53	**0.00024**	84.82	**<0.0001**	14.87	0.094

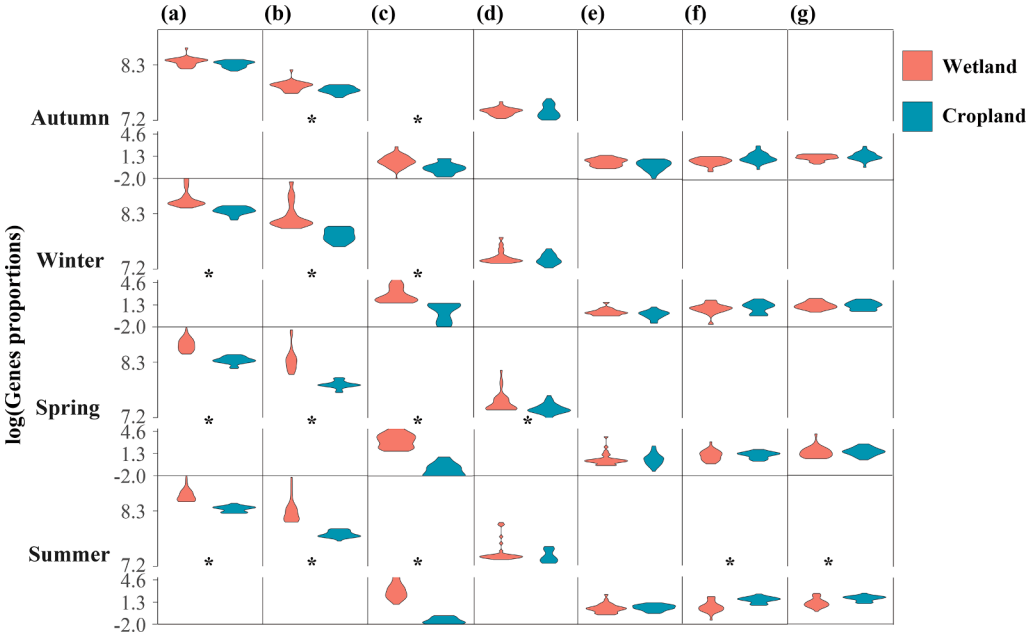

Fig. 3. Effect of cultivation on methane-cycling genes in different seasons. (a). the proportion of total methane related genes; (b). the proportion of methanogenesis genes; (c). the proportion of *mcr* genes; (d). the proportion of CH_4 oxidation genes; (e). the proportion of *sMMO* (encoding soluble CH_4 monooxygenase) genes; (f). the proportion of *pMMO* (encoding particulate CH_4 monooxygenase) genes; (g). *MMO* means the sum of *pMMO* and *sMMO*. "*" indicates that the differences between wetland and cropland are significant at the level of $P = 0.05$.

wetland soils (Fig. 3d). The proportion of *sMMO* genes was insignificant between wetland soils and cropland soils for all four seasons (Fig. 3e). However, the proportion of *pMMO* genes in cropland soils was significantly higher than wetland soils by 129.19 % in summer (Fig. 3f, Fig. S3f). Lumped *sMMO* and *pMMO* together, the proportion of *MMO* genes in cropland soils was significantly higher by 65.55 % than in wetland soils in summer (Fig. 3g).

3.4. Cultivation impacts on vertical distributions of CH_4-related genes along soil depth

The CH_4-related genes varied along soil profile, and the cultivation impacts on the vertical distribution of those genes were dramatic. The proportions of all genes in the wetland, including total CH_4-related genes, methanogenesis genes, and marker genes such as *mcr*, *sMMO*, *pMMO,* and *MMO*, decreased along soil depth ($P < 0.05$, Table 1 and Fig. 4). In cropland soils, the proportion of methanotroph genes, marker genes of *sMMO*, *pMMO* and *MMO* genes significantly varied along the depth, with the highest in layer 30 – 60 cm, but the proportions of total CH_4-related genes, methanogenesis genes, and *mcr* genes were not significantly different among layers (Table 1 and Fig. 4).

The cultivation effects on CH_4-related genes varied along the soil profile. The proportions of total CH_4-related and methanogenesis genes in cropland were significantly lower than those in wetlands across all layers ($P < 0.05$, Fig. 4a, b). The reductions of both total CH_4-related and methanogenesis genes varied along the depth, from 41.58 % at 0 – 10 cm to 11.94 % at 90 – 100 cm and 52.79 % at 0 – 10 cm to 15.87 % at 90 – 100 cm, respectively (Fig. S4a, b). The proportion of *mcr* genes was significantly lower in cropland soils than in wetland soils in all layers (Fig. 4c). The reductions of *mcr* genes were remarkable among layers, ranging from −89 % in layer 60 – 70 cm to −93 % in layer 90 – 100 cm (Fig. S4c). In contrast, the proportion of methanotroph genes is higher in cropland soils than that in wetland soils from 40 to 70 cm, which increased by 5.36 % ~ 9.24 % in cropland vs wetland (Fig. 4d). For the proportion of *sMMO* gene, there were no significant cultivation impacts among all layers (Fig. 4e, Fig. S4e). However, the proportions of *pMMO* and *MMO* genes in cropland were remarkably higher than those from 20 to 60 cm soils in the wetland ($P < 0.05$, Fig. 4f, g). The variation of *MMO* genes was also slightly different among layers, with the highest in layer 20 – 30 cm (46.50 %) and the lowest in layer 80 – 90 cm (21.51 %) (Fig. S4g).

3.5. Cultivation effects on net CH_4 flux and microbes at the global scale

We further compiled 852 values at the global scale (Table S3), and the results showed that land-use changes significantly decreased CH_4 emission while insignificantly influencing on methanogens and methanotrophs (Fig. 5a, b). Specifically, CH_4 emission decreased 92.85 % in Africa, 92.19 % in Australia, 71.93 % in China, 100.00 % in Europe, 92.70 % in India, 36.16 % in southeast Asia and 53.30 % in USA (Fig. 5a). When considering different transition types, CH_4 emission significantly suppressed in all transition types except the tropical

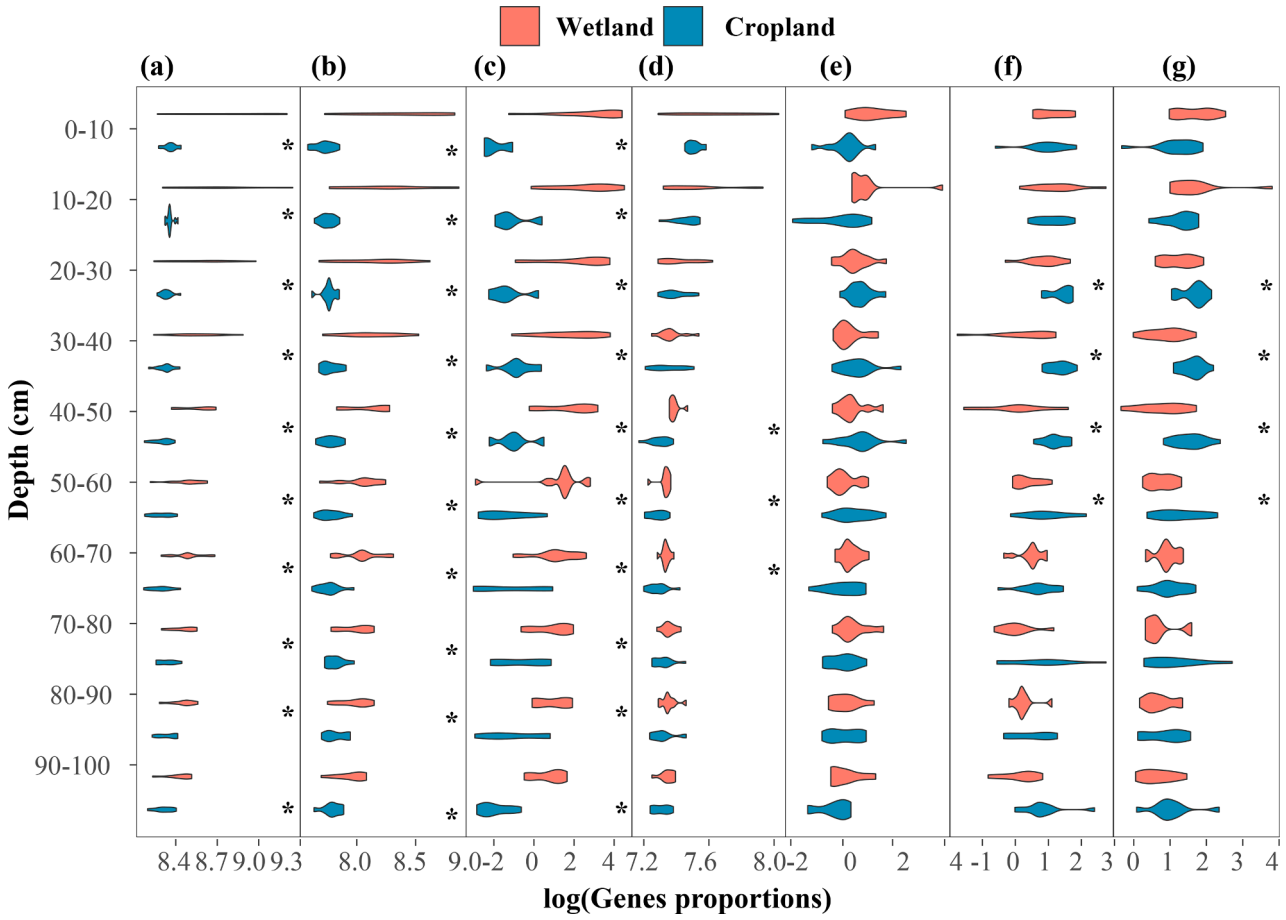

Fig. 4. Response of methane-cycling genes to wetland cultivation in different layers. (a). the proportion of total CH_4-related genes; (b). the proportion of methanogenesis genes; (c). the proportion of *mcr* genes; (d). the proportion of CH_4 oxidation genes; (e). the proportion of *sMMO* (encoding soluble CH_4 monooxygenase) genes; (f). the proportion of *pMMO* (encoding particulate CH_4 monooxygenase) genes; (g). *MMO* means the sum of *pMMO* and *sMMO*. "*" indicates the differences between wetland and cropland are significant at the level of $P = 0.05$.

mangrove converted to the paddy (Fig. S5b). However, the effects of land-use change on microbe-related variables involved in methanogenesis and methanotrophy were insignificant (Fig. 5b). The response ratio showed that methanogens were enhanced in two studies, genes in methanotrophs was suppressed in one study but enhanced in another (Fig. S6b).

3.6. Cultivation impacts on CH_4 cycling

To develop a full picture of cultivation impacts on CH_4 cycling, we summarized our results into a diagram with soil properties, CH_4 processes, and associated functional genes (Fig. 6). The wetland is a strong CH_4 source with an annual budget of 44.93 ± 10.17 g·CH_4 m^{-2}·yr^{-1}, while cropland was a weak CH_4 sink with an annual budget of −0.056 ± 0.051 g CH_4 m^{-2}·yr^{-1}. This source-to-sink transition for CH_4 flux might be attributed to the mechanisms as below (Fig. 6). After 23-years of cultivation, the soil C storage was decreased from 38.29 g·C·kg^{-1} in the wetland to 8.34 g·C·kg^{-1} in the cropland. Along with the C reduction, the proportion of methanogenesis genes, *mcr* genes, and methanotrophic genes significantly declined by 32.10 %, 97.89 % and 7.02 %, respectively, after wetland conversion to cropland. In contrast, the proportion of *pMMO* genes increased by 48.74 % in cultivated cropland soils. Along soil profile, soil total carbon (C), proportions of methanogenesis and *mcr* genes exponentially declined in wetland soils, while methanogenesis and *mcr* genes in cropland soils were similar along the soil profile. Moreover, the proportion of *pMMO* genes was consistent along soil depth in wetland soils while varied along soil profile in cropland soils, which was significantly higher in 20 – 60 cm layers than other layers.

4. Discussion

4.1. Distinct responses of methanogenic and methanotrophic processes to cultivation

Wetland conversion to cropland had distinct impacts on methanogenic and methanotrophic processes. Both CH_4 production potential and net CH_4 flux were inhibited by cultivation (Fig. 1, Fig. 5, Fig S5), along the significant suppression of methanogenesis genes and *mcr* gene, and the stimulation of *pMMO* and *MMO* genes (Fig. 2). It indicates that the source-to-sink transition of net CH_4 flux has resulted from the strong suppression of methanogenesis and the slight stimulation of methanotrophy in soils (Fig. 6), which is consistent with our hypothesis. Thus we infer that the strong suppressions of methanogenesis genes and *mcr* genes, and promotion of *pMMO* genes result in the inhibition of the methanogenesis process and enhancement of the methanotrophic process. In sum, the remarkable decrease in CH_4 production and a slight increase in CH_4 oxidation turn a strong CH_4 source in the wetland into a weak CH_4 sink in cropland (Fig. 6).

The substantial decreases of methanogenesis genes and slight increases of methanotrophic genes suggested that methanogenesis and methanotrophy respond differently to cultivation. This is also partly confirmed by *meta*-data (Fig. S6b) and other studies (Meyer et al., 2017; Zhang et al., 2019), in which found a suppression of methanogenesis while enhancement of methanotrophy. However, both Zhang et al.

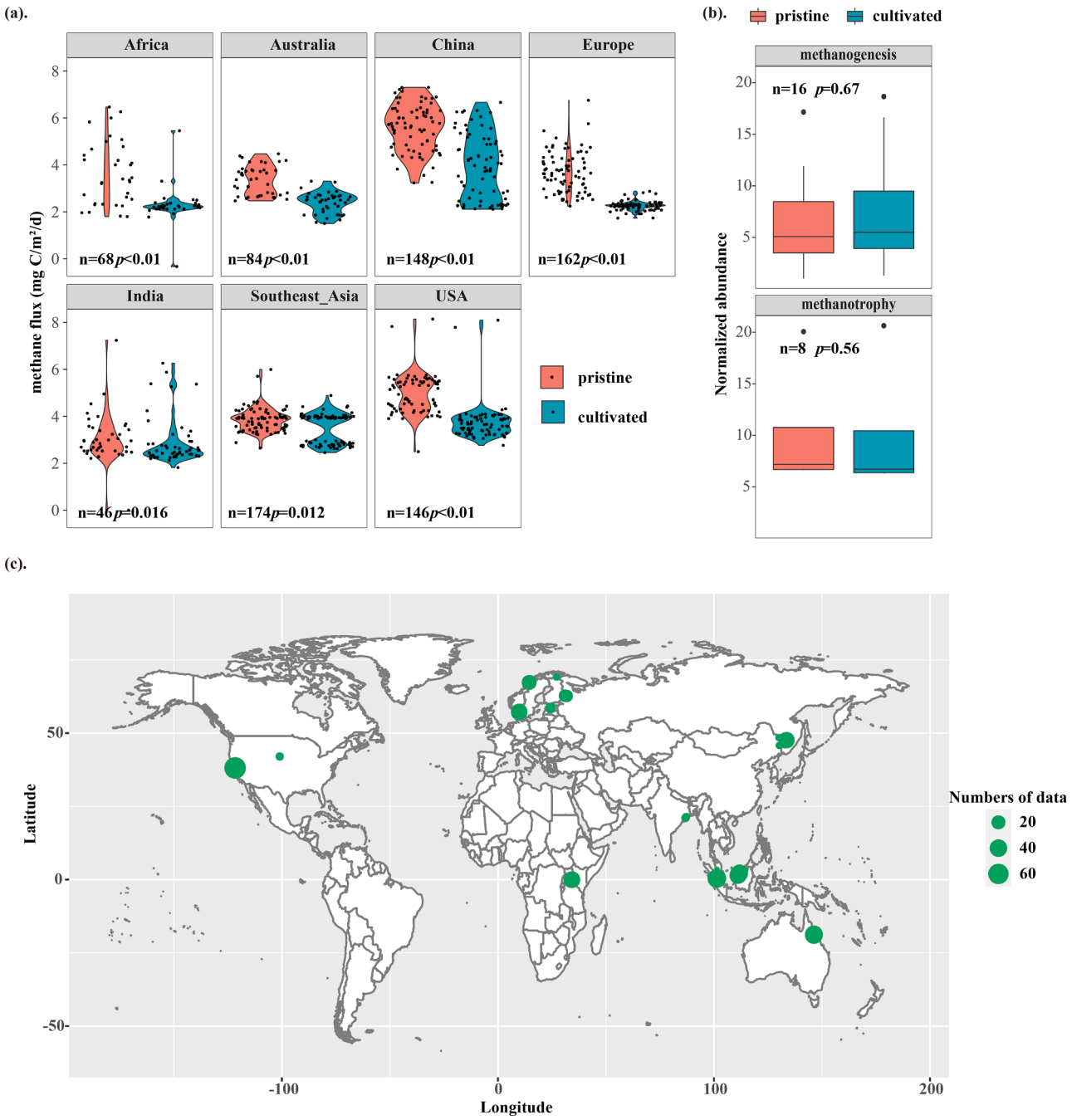

Fig. 5. Effects of land-use transition on methane flux and microbes involved in methanogenesis and methanotrophy. (a). response of methane flux to land-use changes in 7 regions; (b). response of microbes involved in methanogenesis and methanotrophy; (c). location of study sites. Log transformations were used and all variables of methane flux plus 10 to avoid negative values.

(2019) and Meyer et al. (2017) found a stronger response of methanotrophs than methanogenesis to cultivation, while the suppression of methanogens and *mcrA* genes were also found in a study of paddy-soybean conversion (Liu et al., 2018). Methanogenesis is a strict anaerobes process while methanotrophy prefers oxygen, so the methanogenesis would suppress but not methanotrophy when wetland conversion to upland along with decreasing water content and anaerobic–aerobic transition (Lyu et al., 2018; Serrano-Silva et al., 2014). Soil texture and substrate content would be also influenced by land use type, and then affect both methanogens and methanotrophs. The inconsistencies of the environmental factors such as water content, aeration, and soil texture after cultivation bring large uncertainties in the response pattern of methanogens and methanotrophs.

The methanotrophic process includes two steps: CH_4 oxidation to formaldehyde and formaldehyde assimilation, and these two steps respond differently to cultivation. In our study, the *pMMO* and *MMO* gene proportions, which encode CH_4 monooxygenase, increased after cultivation (Fig. 2f, g). In contrast, the proportion of methanotrophic genes involved in formaldehyde assimilation pathways decreased (Fig. 2d). The inconsistent response of *pmoA* abundance and methanotrophs to dry and wet seasons were reported in a tropical wetland, in which *pmoA* abundance increased while methanotrophs decreased in dry seasons (Gontijo et al., 2020). (Fig. S2), Moreover, the proportion of *pMMO* genes to total *MMO* and the enhancement of cultivation to *pMMO* genes were larger than that of genes for *sMMO* (Fig. 2), which indicated *pMMO* was dominant in response to cultivation in our study site. *pMMO*,

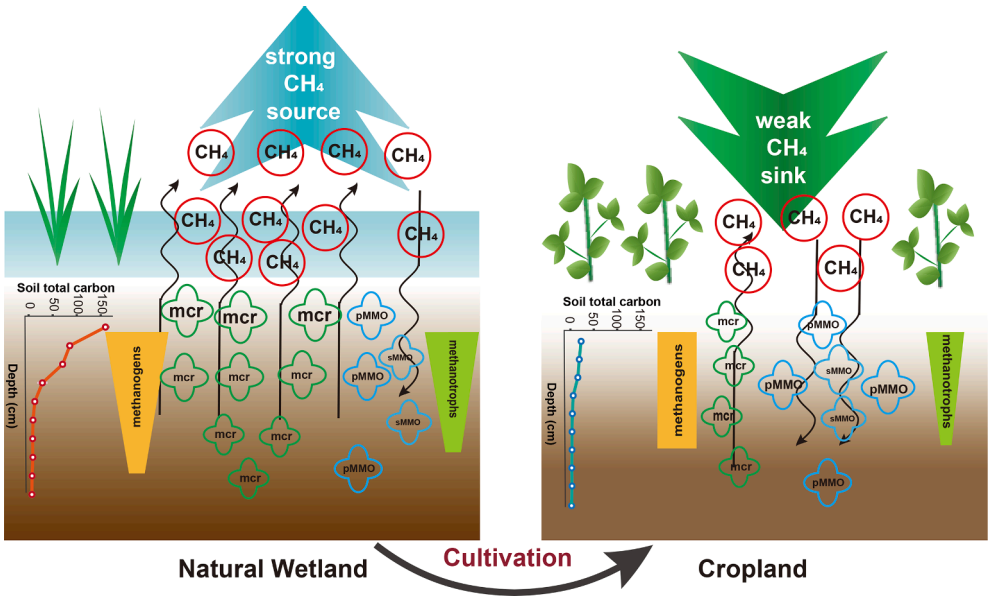

Fig. 6. Thematic diagram illustrating the biogenic mechanisms of cultivation impacts on CH_4 emission. (After wetland conversion to cropland, soil organic C decline causes remarkable inhibition of methanogens and *mcr* genes while slight stimulation of *pMMO* genes, resulting in a transition from a CH_4 source to a CH_4 sink).

which is expressed by all but one genus of methanotrophic bacteria, was much more prevalent than *sMMO*, which is produced by a small subset (Dumont and Murrell, 2005; Hakemian and Rosenzweig, 2007). Thus, the dominance of *pMMO* in methanotrophs and the significant enhancement of *pMMO* confirmed the increase of methanotrophic process after wetland cultivation. However, the significant enhancement of CH_4 oxidation rate was not confirmed on a global scale due to data scarcity. The inconsistency of functional genes with CH_4 oxidation rate would impede exploring the underlying mechanism, and also calls for further work to estimate CH_4 oxidation potential in wetlands.

4.2. Seasonal patterns in methanogenesis and methanotrophic pathways

Seasonal patterns of CH_4-related genes are different in wetland and cropland. In the wetland, total CH_4-related genes, methanogenesis and *mcr* genes varied substantially across four seasons, with the highest in spring or summer and the lowest in autumn. It is consistent with the fluctuations of temperature in our sampling sites, high in summer and dropping to zero in autumn (Fig. S2a). It also consistent with the seasonal pattern of soil water content, which is higher in summer than in autumn (Wang et al. 2022). In comparison, the genes related to methanotrophy including *sMMO*, *pMMO* and *MMO* in the wetland, were not significantly different across seasons (Fig. 3, Table 1). The genes fluctuations were also similar to the CH_4 emission in the Sanjiang wetland, which represented temporal patterns with peak values in summer (June and August) (Zhu et al., 2014). Previous studies showed that CH_4 emissions and microbial communities changed at diurnal and seasonal scales (Bansal et al., 2018; Gontijo et al., 2020; Martinez-Cruz et al., 2015). The variations in the water table, soil temperature, and water-filled pore space of soil together can explain 66.7 % of the observed temporal variation of the net CH_4 fluxes (Zhu et al., 2014). The same temporal pattern of CH_4 emission was also observed in a peatland in northern America, primarily caused by temperature as well as hydrologic dynamics (Feng et al., 2020). Hence, the temperature might be important in driving seasonal variation of CH_4-related genes thus the net CH_4 flux in the wetland.

Seasonal patterns in methanogenesis and methanotrophic genes varied after cultivation. In cropland, the proportions of total CH_4-related genes, methanogenesis genes, and methanotrophic genes in the cropland among seasons change insignificantly (Fig. 3, Table 1), while larger changes of soil temperature occurred in the cropland than in the wetland (Fig. S2b). These results suggest that soil temperature was not the main factor controlling functional genes variation in the cropland. Compared with wetlands, the variations of methanotrophic genes were also conservative across seasons (Fig. S3). However, the marker genes of *mcr*, *pMMO*, and *sMMO* genes were significantly shifted across seasons in the cropland (Table 1). Those results indicate that marker genes and the genes involved in whole metabolism pathways, such as CH_4 production and oxidation, present different seasonal patterns in the cropland, which are worth further exploration. Moreover, besides soil temperature, other environmental factors, such as soil organic matter, pH, and CH_4/O_2 ratio, would also impact seasonal patterns of functional genes (Tate, 2015). The mechanisms under these different seasonal patterns between wetlands and cropland required further investigation.

4.3. Vertical distributions of methanogenesis/methanotrophic genes along soil depth

The vertical distributions of CH_4-related genes differed between wetland and cultivated cropland. In our study, the proportions of all CH_4-related genes significantly decreased along soil depth in the wetland (Fig. 4, Table 1). The consistent results in bacteria and fungi based on the PLFAs and microbial biomass were also observed in both regional experiments and global syntheses (Fierer et al., 2003; Xu et al., 2013; Zhu et al., 2021), which can be resulted from declining resource availability throughout the soil profiles (Jerman et al., 2017; Zhu et al., 2021). However, proportions of total CH_4-related genes, methanogenesis and *mcr* genes did not vary significantly in cropland (Fig. S3, Table 1). Homogenization of microbial properties, including soil biomass C, nitrogen, and phosphorus, was observed after 23-year cultivation in our previous study (Zhu et al., 2021). This homogenization of soil microbial properties and functional genes caused by mixing process of cultivation could result in the downward movements of soil C and nutrients from topsoil. Moreover, even without tilling, a significant decrease of nutrients input at the soil surface would be also remarkable because of harvesting seeds every year.

The cultivation impact on methanogenesis and methanotrophic marker genes varied along soil profiles. For instance, the decreasing effects of cultivation on the proportion of methanogenesis genes and *mcr* genes were more severe in shallow soil layers (0 – 30 cm) than in deep

soil layers (Fig. S4). However, higher proportions of *pMMO* and *MMO* genes were observed in middle layers (30–70 cm) in cropland soils (Fig. 4, Fig.S4). Similar patterns were also observed in bacterial concentrations based on PLFAs (Zhu et al., 2021). These differences could be explained by the distinct response patterns of the dominant microbial group involved in methanogenesis and methanotrophs, mainly archaea and bacteria. Archaea involved in methanogenesis are O_2 sensitive, while the group of methanotrophs attributed to bacteria communities are reliant upon soil availability of carbon and O_2 (Evans et al., 2019; Hanson and Hanson, 1996; Nagler et al., 2020). Reduced carbon and increased O_2 in surface layers in cropland contribute to the declines in *mcr* genes and increases in *pMMO* and *MMO* genes. Taken together, soil nutrients and O_2 play major roles in distinct vertical distributions of functional genes related to CH_4 cycling in wetland and cropland soils (Jiao et al., 2018).

4.4. Limitations and prospects

The CH_4 processes and associated functional genes were examined in this study to develop a comprehensive understanding of a transition from a CH_4 source to a CH_4 sink after wetland conversion to cropland. We identified three drawbacks as follow-up work to advance our understanding. First, the duration of cultivation, as well as restoration, is a vital factor that influences the responses of methanogens and methanotrophs, which was excluded in the present study. Therefore, a time series sampling would help refine the full picture of cultivation impacts on CH_4 cycling. Second, different measurement methods would reflect different aspect characteristics of soil microbes involved in the CH_4 cycling. Thus, understanding the CH_4 cycling from various perspectives of phylogeny, function trait, and metabolism activity would be served as a foundation for a solid understanding. Third, long-term monitoring of climatic factors, physiochemical characteristics, and microbes along soil profile would provide a comprehensive insight into ecosystem CH_4 cycling and its underlying mechanisms.

This study is among the first attempts to comprehensively investigate the CH_4 processes and methanogenesis and methanotrophic genes in four seasons along soil profiles, although a large number of studies have investigated the impacts of land-use change on soil microbial communities associated with CH_4 cycling (Gao et al., 2020; Gao et al., 2021; He et al., 2015). The integration of geochemical and metagenomic data in this study deduces the CH_4 cycling and advances our understanding of microbial mechanisms for CH_4 processes. It also provides insights into microbial controls over CH_4 cycling along soil profiles and across seasons, which are the essential data for the incorporation of microbial mechanisms into microbial models for better simulating CH_4 flux. It should be noted that although this study confirmed the ecological benefit of wetland conversion to cropland for CH_4 sink, the valuable roles of wetlands in carbon sequestration and providing ecosystem services are substantial and should not be disregarded when evaluating the ecological consequences of wetland conversion to cropland.

5. Conclusions

In this study, we examined the CH_4 processes, and the functional genes involved in methanogenesis and methanotrophy and their biomarker genes along soil profiles across four seasons. The proportion of methanogenesis and methanogenesis marker genes - *mcr* genes is largely suppressed by pristine wetland cultivation, while the proportion of *pMMO* slightly increased in cropland, particularly in summer and middle soil layers from 20 to 60 cm, which led to the reduction of CH_4 emission. The decrease of methanogenesis and *mcr* genes after cultivation was significant in four seasons, while the increase of *sMMO*, *pMMO* and *MMO* genes were only remarkable in spring or summer. A global *meta*-analysis confirmed the remarkable reduction of CH_4 emission after wetland cultivation and its underlying microbial mechanisms. Our results provide insight into the distinct response patterns of functional genes involved in methanogenesis and methanotrophy to cultivation based on the metagenomic analysis and lay a foundation for climate mitigation by managing soil microbial processes.

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.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2022.116229.

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