Science of the Total Environment 792 (2021) 148292

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Soil aeration rather than methanotrophic community drives methane uptake under drought in a subtropical forest

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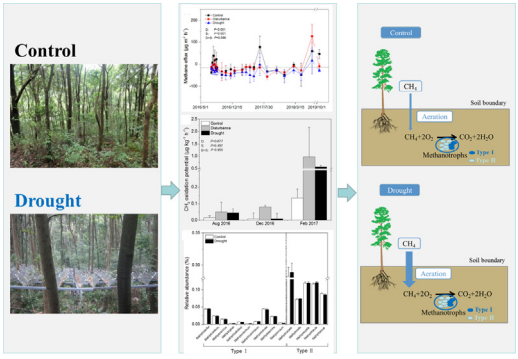
HIGHLIGHTS

- Meta-analysis found that CH_4 uptake under drought in subtropical forests is understudied.
- We took 3-year *in situ* CH_4 efflux measurements under drought in a subtropical forest.
- Drought did not change the oxidation potential and abundance of methanotrophs in soils.
- Soil aeration rather than methanotrophs drives soil CH_4 uptake in subtropical forests.
- Active methanotrophic communities were dominated by the genus *Methylosinus* in soils

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 20 March 2021
Received in revised form 26 May 2021
Accepted 1 June 2021
Available online 5 June 2021

Editor: Jay Gan

Keywords:
Drought
CH_4 uptake
Methanotrophic activity
Methanotrophic community
Stable isotope probing
Forest biome

ABSTRACT

Little information is available about the effects of drought on soil methane (CH_4) uptake and the underlying feedback of the soil microbial community in forest biomes. More importantly, a meta-analysis of the current literature on this topic revealed that there are virtually no data available in subtropical forests. To fill the abovementioned knowledge gap, we carried out a 3-year investigation of *in situ* CH_4 efflux under drought in a subtropical forest, and found that drought significantly increased soil CH_4 uptake ($P < 0.001$). However, drought did not change oxidation potentials and abundances of methanotrophs, and similar methanotrophic communities were observed between the drought and ambient control sites based on metagenomic sequencing analysis. Active methanotrophic communities were dominated by the genus *Methylosinus* based on DNA stable-isotope probing analysis. Structural equation model analysis indicated that direct drought-derived pathway, *i.e.*, increasing soil aerations, outweighs the indirect pathway, *i.e.*, altering methanotrophic communities and activities, and plays a predominant role in driving soil CH_4 uptake in forest ecosystems. To our knowledge, our work is the first study to investigate the effects of drought on *in situ* CH_4 efflux and the underlying microbial mechanisms in subtropical forests.

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https://doi.org/10.1016/j.scitotenv.2021.148292
0048-9697/

1. Introduction

It is predicted that extreme climatic events such as drought will occur with greater frequency and longer duration by the end of this century (IPCC, 2013). Drought has dramatic impacts on forest ecosystem functioning, inducing tree mortality and reducing forest ecosystem functions. One particular process that will be very vulnerable to drought is soil methane (CH_4) uptake (Wang et al., 2012; Anderegg et al., 2013; Schlesinger et al., 2016). Approximately 25% of the global warming potential is driven by CH_4, making it the second most important greenhouse gas after carbon dioxide (CO_2) (IPCC, 2013). Given that forest ecosystems are the largest terrestrial CH_4 sink, oxidizing ~14.2 ± 15.5 Tg CH_4 per year from the atmosphere (Dutaur and Verchot, 2007), CH_4 uptake in forest soils has consequently received considerable attention (Wang et al., 2014; Tate, 2015; Ni and Groffman, 2018). However, the microbial dynamics underlying CH_4 uptake are still poorly understood. This knowledge gap greatly hinders our understanding of CH_4 oxidation in forest soils, its potential sensitivity to climate change (Ni and Groffman, 2018) and the development of a suitable global CH_4 cycling model (Kolb, 2009; Tate, 2015), particularly in the context of extreme climate conditions (Schlesinger et al., 2016).

The oxidation of CH_4 within forest soil is mediated by a specific group of microorganisms known as methanotrophs (Hanson and Hanson, 1996; Kolb, 2009). Methanotrophs are Gram-negative bacteria within the Verrucomicrobia, Proteobacteria, and the novel phylum NC10 (Bowman, 2011). On the basis of their physiological, biochemical and morphological properties, methanotrophs have been classically divided into Type I methanotrophs (Gammaproteobacteria, *Methylococcaceae*) and Type II methanotrophs (Alphaproteobacteria, *Methylocystaceae*) (Hanson and Hanson, 1996). The discovery of new methanotrophs has challenged this classification but it is still used in this study, since current phylogenies reflect this classification (Dedysh and Yilmaz, 2018). The key step in soil CH_4 oxidation is catalyzed by CH_4 monooxygenase, which converts CH_4 into methanol in the CH_4 oxidation pathway. The monooxygenase enzyme has two forms, the particulate membrane bound form and the soluble form (Hanson and Hanson, 1996; Kolb, 2009). As most methanotrophs have the particulate membrane bound monooxygenase enzyme, the *pmoA* gene, which encodes the β-subunit of the particulate membrane bound monooxygenase enzyme, has been widely used as a biomarker to investigate methanotrophic communities in terrestrial ecosystems (Gu et al., 2019; Fest et al., 2015).

Previous studies have demonstrated that soil moisture content is one of the most important environmental factors influencing CH_4 oxidation rates (Tate, 2015). Through a literature review, we compiled a schematic graph to show how drought can influence soil CH_4 uptake *via* direct and indirect pathways in forest ecosystems (see Fig. 1). On the one hand, drought can increase soil aeration, thereby increasing the availability of CH_4 in the soil gas atmosphere and stimulating soil CH_4 oxidation (Fest et al., 2015). On the other hand, drought can have strong inhibition effects on methanotrophic activities (Knief et al., 2005; Zhou et al., 2008a; Nazaries et al., 2011; Tate, 2015). Previous studies have shown that soil CH_4 oxidation is linked to changes in the community structure and the abundance of methanotrophs (Tate, 2015). Some studies have stated that methanotrophic abundance could act as a useful tool for predicting changes in methanotrophic activity (Nazaries et al., 2011, Tate, 2015). Therefore, we expect that declines in methanotrophic abundance would decrease soil CH_4 oxidation in forest ecosystems (Fig. 1). In addition, we could use stable isotope techniques to detect the active methanotrophic communities that contribute to soil CH_4 oxidation (Dumont et al., 2011; Cai et al., 2016).

In this study, we aimed to find evidence for the proposed direct and indirect pathways underlying our conceptual model (Fig. 1). First, we carried out a meta-analysis to quantify the effects of drought on *in situ* soil CH_4 efflux across forest biomes worldwide. To our surprise, there were no studies on the effects of drought on *in situ* soil CH_4 efflux in subtropical forests available. In addition, all published work did not focus on methanotrophic community dynamics. Therefore, we conducted a long-term drought experiment to investigate the responses of 3-year *in situ* soil CH_4 efflux and the associated methanotrophic community dynamics to drought in a subtropical evergreen forest. Finally, we quantified the relative contributions of the direct and indirect drought-derived pathways to soil CH_4 efflux in forest ecosystems.

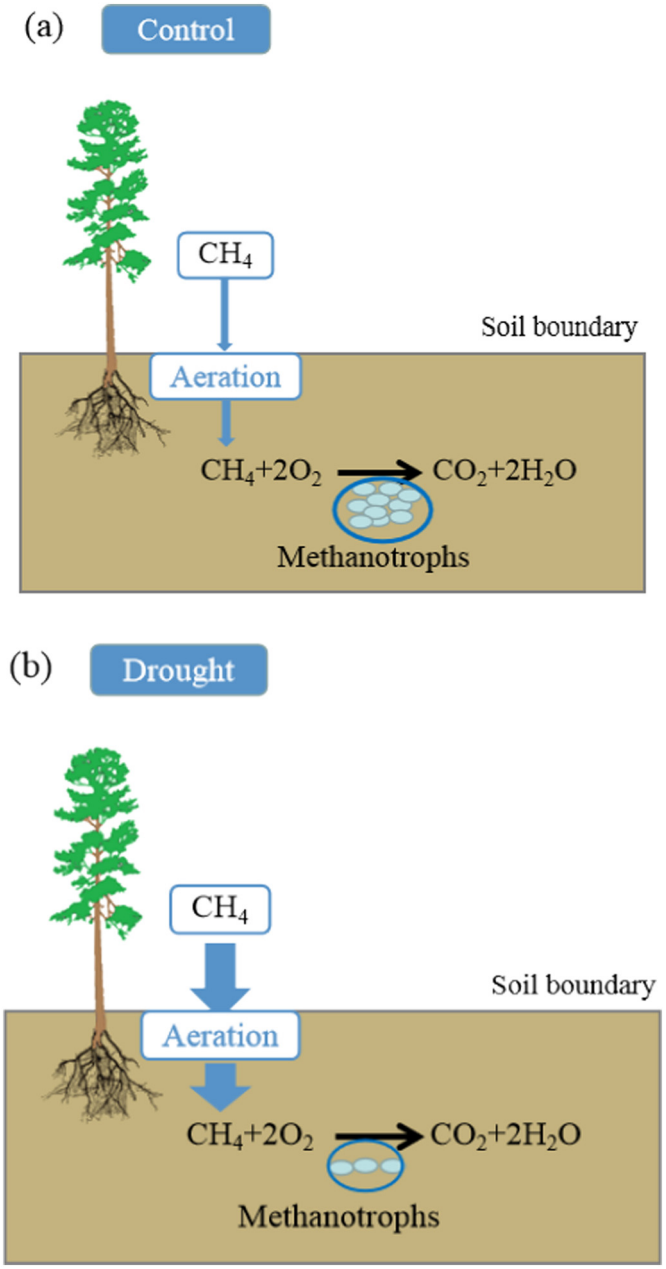

Fig. 1. Schematic graph showing variations in soil CH_4 oxidation under drought in a subtropical evergreen forest. Methanotrophs in aerobic conditions can convert CH_4 to CO_2 through methanotrophic activity (a). The positive effect of drought is the increase in soil aeration, thereby increasing the availability of CH_4 to the soil gas atmosphere, thus stimulating CH_4 oxidation; however, the negative effect of drought is that methanotrophic communities could be greatly changed under drought, thereby inhibiting soil methanotrophic activity, thus reducing CH_4 oxidation (b).

2. Materials and methods

2.1. Meta-analysis of the effects of drought on soil CH_4 uptake across forest biomes

We systematically searched for peer-reviewed journal articles in the Web of Science database with the following search term combinations:

[drought OR throughfall reduction OR rainfall manipulation OR reduced precipitation] AND [methane flux OR CH_4] AND [forest]. We reviewed all the articles revealed through the search and selected those that met the following two criteria: (1) the study was conducted on *in situ* field measurements for at least 4 months; (2) soil CH_4 uptake could be extracted directly from the text, tables and figures. When one publication included several experiments under different abiotic conditions, such as different locations, tree species or stand ages, we considered them to be different observations. In total, we found 28 datasets for forest soils from 20 sites in 13 papers (Table S1). All of the studies used the automated or manual static chamber methods. For each selected study, we collected the latitude, longitude, mean annual temperature and mean annual precipitation. We also collected information on drought intensity, calculated from the percentage by which rainfall was reduced, treatment duration and climate zones. We used GetData to digitally extract the data from figures when the results were reported graphically. Control and treatment means, standard deviations and sample sizes (n) of CH_4 were directly extracted or recalculated, and CH_4 uptake rates were standardized to mg CH_4 m^{-2} day^{-1}.

Defined as the "effect size", the natural log of the response ratio (ln R) was used to assess the responses of CH_4 uptake to the treatments. We calculated the response ratios from each study *via* the methods described by Zhou et al. (2017) and Feng et al. (2020). Briefly, ln R was calculated as follows:

$$\ln\left(X_i/X_n\right) = \ln X_i - \ln X_n \quad (1)$$

where X_i and X_n are the values of each observation in the treatment and the corresponding control plots, respectively. The sampling variance for each ln R was calculated as:

$$\ln\left[(1/n_i)\times(S_i/X_i)^2 + (1/n_n)\times(S_n/X_n)^2\right] \quad (2)$$

where n_i and n_n, S_i and S_n, and X_i and X_n are the treatments and control samples' sizes, standard deviations and mean responses, respectively. The effects on CH_4 uptake and the differences between the treatment and control plots were considered to be significant if the 95% CI of ln R did not overlap zero.

2.2. Measurements of soil CH_4 uptake over 3 years under drought in a subtropical forest

We selected a long-term drought platform for the rainfall reduction manipulation experiment, which was established in July 2013 at Tiantong National Forest Park, Ningbo, Zhejiang Province, Eastern China (29°52′N, 121°39′E, 200 m above sea level). The region is dominated by a typical subtropical monsoon climate, with a mean annual temperature of 16.2 °C. The mean annual precipitation is 1383.94 mm (1953–2012). The dominant tree species at the study site are *Castanopsis fargesii* Franch., *Schima superba* Gardner and Champ., *Castanopsis carlesii* (Hemsl.) Hayata and *Lithocarpus glaber* (Thunb.) Nakai (Bu et al., 2018; Wang et al., 2020). We manipulated the site with three treatments: a 70% rainfall reduction to simulate an extreme drought scenario in the future (hereafter referred to as the "drought" treatment) by using large plastic plates, a shade treatment to account for the effect of the plates in the drought treatment (hereafter referred to as the "disturbance" treatment) and an ambient control treatment. Each treatment had three replicates and the size of each plot is 25 m × 25 m, with at least 5 m spacing between adjacent plots. In the drought plots, transparent concave polycarbonate plates were uniformly fixed at a height of 1.5–3.5 m above the ground to reduce rainfall. We installed identical plates in the disturbance plots but the grooves of these plates were placed face down to allow rainfall to penetrate, whereas these plates were placed face up in the drought plots. Detailed information about the experimental site and the soil properties are given in Table S1 and in previous studies (Bu et al., 2018).

For the *in situ* CH_4 efflux measurements, we collected samples between 9:00 am and 12:00 am local time from all plots over 3 years from July 2016 to October 2019: first once a week between July 2016 and August 2016, then once a month between September 2016 and July 2017, and finally once every 2 months between July 2017 and July 2018 and once in October 2019. Measurements of CH_4 efflux were carried out *via* the static chamber approach (Zhou et al., 2008b; Stiles et al., 2018). The chambers were made from polyethylene and consisted of removable cover boxes (30 cm in diameter and 40 cm high, without a bottom), equipped with a three-way sampling port and a cylinder collar. For each efflux measurement, gas samples (~10 mL) were taken with a 10-mL polypropylene syringe at 10-min intervals over 40 min after deployment. The chamber's inner temperature, the ambient air temperature and the soil temperature at a depth of 5 cm were measured manually with a handheld digital thermometer after the last efflux measurement. Soil moisture in the upper 10 cm of the soil was recorded with a hand-held TDR sensor (FieldScout TDR 100, Spectrum Technologies, USA). The samples were then transported in glass vials to the laboratory and subsequently analyzed on a gas chromatograph (Agilent 7890B GC, USA) within 48 h. The chromatograph was equipped with a flame ionization detector to analyze the CH_4 concentration; the carrier gas was N_2. Methane flux was calculated from the slope of a linear regression between gas concentration and sampling time (Zhou et al., 2021).

Additionally, we collected soil samples twice: once in early August 2016 and again in early December 2016. We used a soil auger (2.5 cm in diameter) at a depth of 0–10 cm within each plot following a diagonal sampling pattern (*i.e.*, one point at each corner and one in the center of each plot). The soil cores were immediately mixed thoroughly and kept in a cooler at 4 °C. The samples were then passed through a 2-mm sieve to remove roots and stones, and stored at 4 °C before further analysis. Subsamples were stored at −20 °C for DNA extraction.

2.3. Measurements of oxidation potential, abundance, community structure and active taxa of methanotrophs under drought in a subtropical forest

All the soil samples were incubated in the laboratory to measure their CH_4 oxidation potential (*i.e.* methanotrophic activity) with ambient air containing ~2 parts per million (ppm) CH_4. About 10 g (dry weight equivalent) of field-moist soil was incubated in a 1-L sealed flask under ambient CH_4 in the dark at 22 °C for 7 days. Headspace gas samples (approximately 10 mL) were collected through a rubber septum in the jar lid at the beginning and the end of the incubation, and the concentrations of CH_4 were determined *via* gas chromatography (GC7890B, Agilent). The CH_4 oxidation rates in each flask were calculated from differences in the headspace CH_4 concentration over the incubation time (Zhou et al., 2018) and adjusted to soil dry weight. Standards were measured once every 10 samples; the coefficient of variation in CH_4 oxidation potential was less than 5%.

For measurements of methanotrophic abundance and community structure, soil genomic DNA was extracted from soil samples according to procedures described previously (Zhou et al., 2010; Bu et al., 2018; Gu et al., 2019). The abundance of methanotrophs was quantified *via* real-time quantitative polymerase chain reaction (PCR) targeting the *pmoA* gene with the primers A189F (forward) and A650R (reverse), as described previously (Gu et al., 2019). All real-time quantitative PCR reactions were performed with the ViiA 7 Real-Time PCR System (Applied Biosystems, CA, USA).

Methanotrophic communities were analyzed *via* two methods: high-throughput sequencing and metagenomic analysis. For high-throughput sequencing, universal bacterial *16S* rRNA primers of 515F and 907R were used to target soil genomic DNA samples in this subtropical forest (Bu et al., 2018). The oligonucleotide sequences included a 12-bp barcode fused to the forward primer. The PCR amplification procedure has been described previously elsewhere (Bu et al., 2018). The amplicons were sequenced on an Illumina Miseq in PE250 mode

(Illumina, Nanjing, China). The QIIME and UPARSE software tools were used to analyze the amplicons' sequencing data. Paired-end data from each sample were joined with FLASH with the default parameters, as described previously (Bu et al., 2018). Operational taxonomic units were clustered at a similarity of 97%. For the metagenomic analysis of soil genomic DNA, the total DNA extracted from the soil samples of the control and drought plots in December 2016 was sequenced with an Illumina HiSeq 4000 (Illumina). This produced an average of 91.78 Mb of high-quality reads for each sample, providing a total of 275 Mb of read data. The high-quality reads were assembled *de novo* into contigs with Megahit version 1.0.6 (Li et al., 2015), with the default parameters for all samples. The contigs with lengths of more than 500 bp in MetaGeneMark (version 3.26) were used and a nonredundant gene catalogue was constructed by CD-HIT with a sequence identity cutoff of 0.95 and a minimum coverage cutoff of 0.9 for the contigs (Li and Godzik, 2006). Gene taxonomy classifications in our catalog were determined by searching against the NCBI-NR database (June 2017 version) (Xue et al., 2020). Functional annotation of the protein sequences of the predicted genes were determined by searching against the Kyoto Encyclopedia of Genes and Genomes (KEGG) database with DIAMOND blastp and an e-value threshold of 10^{-5} (Buchfink et al., 2014).

Additionally, to detect the active methanotrophs in this forest, we selected soil samples from only the control and drought plots in December 2016 for DNA-SIP analysis. In brief, approximately 10 g (dry weight equivalent) of field-moist soil was incubated in a 1-L sealed flask under ambient air and 2000 ppm $^{13}CH_4$ in the dark at 22 °C for 3 weeks for $^{13}CH_4$ labelling. During this period, we monitored the changes in $^{13}CH_4$ concentration in the headspace once a week to make sure that the $^{13}CH_4$ had been used up by the methanotrophs as a substrate. After incubation, total genomic DNA was extracted as described above. DNA-SIP fractionation was performed as described previously (Gu et al., 2019; Zheng et al., 2014). Up to 14 gradient fractions were generated, and we selected the heavy fraction (the fourth, fifth and sixth fractions) of the fractionated DNA gradient for sequencing (Cai et al., 2016). A refractive index measurement of each fraction was determined with an AR200 digital hand-held refractometer (Reichert, Inc., Buffalo, NY, USA). The fractionated DNA was recovered by PEG-6000 precipitation and re-suspended in 30 μL of a TE buffer, and then used for Illumina MiSeq sequencing of the *16S* rRNA as described above.

2.4. Statistical analysis

For the meta-analysis of the effects of drought on soil CH_4 uptake, a categorical random effect model was used to assess whether CH_4 uptake showed different responses to different drought intensities, durations and climate zones. All statistical analyses were performed in MetaWin 2.1.

Additionally, repeated-measure (two-way) ANOVA was used to determine the effects of drought and sampling time on *in situ* CH_4 efflux, and the activity and abundance of methanotrophs in the subtropical forest. A generalized additive model was used to assess the correlations between *in situ* CH_4 effluxes. Differences were considered significant at $P < 0.05$.

Furthermore, structural equation modeling was performed using R software with the lavaan R package to explore the causal links among *in situ* soil CH_4 efflux; soil moisture contents; soil properties such as pH, soil total C and N contents, soil NH_4^+–N and NO_3^-–N contents; and methanotrophic community and abundance in the subtropical forest. Paths in this model were considered to be significant at $P < 0.05$ (Zhou et al., 2017). All analyses were performed in R (R Core Team, 2014).

2.5. Accession numbers

All raw sequence data from this study have been deposited in the DNA Data Bank of Japan (DDBJ) biosample database with the accession numbers PRJDB7138 and PRJDB9136.

3. Results

3.1. Effect of drought on soil CH_4 uptake across forest biomes

On average, drought significantly increased soil CH_4 uptake rates by 44%, with the 95% confidence interval (CI) being between 0.31 and 0.41 (Fig. 2). To examine the effects of drought and climate components on soil CH_4 uptake across forest ecosystems, we grouped all these datasets into different drought intensities (low intensity, <30% reduction in rainfall; moderate intensity, 30–60% reduction in rainfall; high intensity, >60% reduction in rainfall), drought duration periods (short term, <1 year; moderate term, 1–2 years; long term, >2 years) and climate zone (temperate, subtropical and tropical). We found that irrespective of the intensity, drought significantly increased soil CH_4 uptake, particularly under high drought intensity (102%; 95% CI: 0.58–0.83) (Fig. 2). Similarly, drought significantly increased soil CH_4 uptake by 36–52% irrespective of drought duration. To our surprise, we only found that drought increased CH_4 uptake by 34% (95% CI: 0.24–0.34) and 101% (95% CI: 0.58–0.82) in temperate and tropical forests (Fig. 2), respectively. Nevertheless, we did not find any data about the response of soil CH_4 uptake to drought in subtropical forests.

3.2. Variations in in situ CH_4 efflux under drought in a subtropical forest

To fill the abovementioned knowledge gap, we carried out a 3-year investigation of *in situ* CH_4 efflux under drought in a subtropical forest. We found that drought plots had significantly lower soil CH_4 efflux

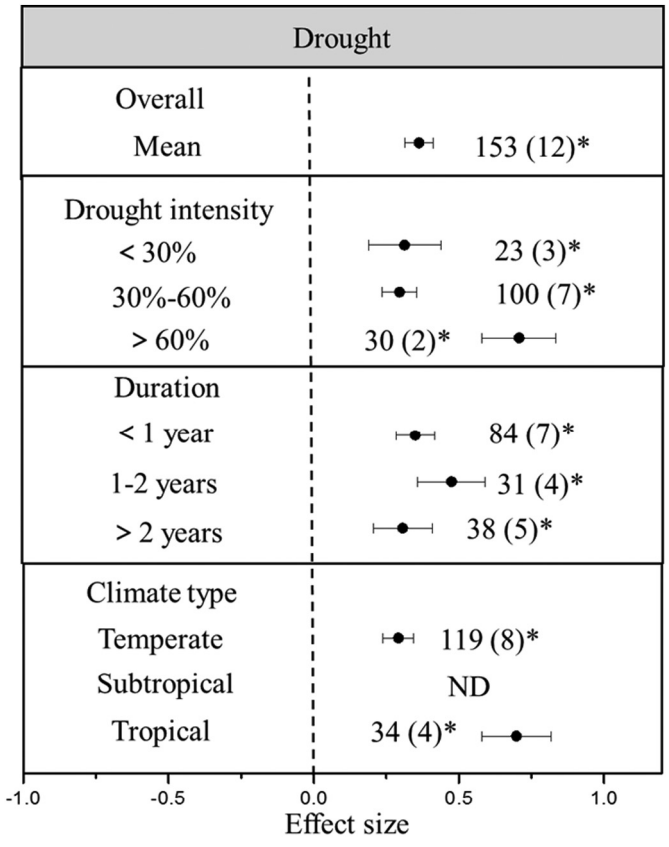

Fig. 2. The mean effect sizes of throughfall reduction on CH_4 uptake in forest soils. The data are categorized into different groups on the basis of drought intensity, treatment duration and climate zone. Error bars represent 95% confidence intervals. The dashed line is drawn at mean effect size = 0. The effect was considered significant if the 95% confidence interval of the effect size did not cover zero. *$P < 0.05$. Number values for each bar indicate the sample size and numbers in brackets indicate the number of experiments. ND, not detected.

than the ambient control plots ($P < 0.001$, Fig. 3a), although soil CH_4 efflux varied considerably in this subtropical evergreen forest, ranging from -55 ± 23 μg m^{-2} h^{-1} to 126 ± 56 μg m^{-2} h^{-1} across the sampling times (Fig. 3a). All plots acted as CH_4 sinks, except for the plot sampled in July 2018, and the control plot acted as a CH_4 source in July 2016, July 2017 and October 2019.

Estimates of annual CH_4 efflux from July 2016 to July 2017 indicated that all sites acted as net soil CH_4 sinks (Fig. 3b). Drought (-388 ± 52 mg CH_4 m^{-2} yr^{-1}) significantly increased soil CH_4 uptake compared with the control treatments (-203 ± 49 mg CH_4 m^{-2} yr^{-1}) but there were no significant differences in CH_4 efflux between the disturbance and control treatments (Fig. 3b).

3.3. Oxidation potential, abundance, community structure and active taxa of methanotrophs under drought in a subtropical forest

We found that drought had no effects on CH_4 oxidation potential compared with the control treatment when soil samples were incubated at ambient atmospheric CH_4 concentrations (Fig. 4a). Measurements of *pmoA* gene copy numbers determined that drought produced similar soil methanotrophic abundance to the control treatment (a mean value of 6.3×10^4 copies g^{-1} under drought *vs* 6.7×10^4 copies g^{-1} in the control plots; Fig. 4b), which was supported by the abundance of *pmoA* and other key functional genes involved in CH_4 oxidation being similar between the two sites according to metagenomic sequencing analysis (Fig. S2).

High-throughput sequencing of *16S* rRNA revealed similar methanotrophic communities in the drought and control treatments. Methanotrophic communities in soils were dominated by the genera *Methylobacter* (Type I methanotrophs) and *Methylocella* (Type II methanotrophs) (Fig. 5a). Similar results regarding the methantrophic communities between the two sites were observed through metagenomic sequencing analysis (Fig. 5b). Metagenomic analysis detected 15 methanotrophic genera, with the genus with the highest relative abundance being *Methylocapsa*, followed by, in decreasing order *Methylocystis*, *Methylosinus* and *Methylocella* (Fig. 5b). Overall, the methanotrophic communities were dominated by Type II methanotrophs (0.072–0.288% *vs.* 0.002–0.045% Type I methanotrophs) (Fig. 5b). We also found that drought tended to increase the relative abundance of the majority of Type II methanotrophs (Fig. 5b).

Furthermore, we detected active methanotroph communities under drought by using DNA stable-isotope probing (DNA-SIP) analysis. The results showed that active methanotrophic communities were dominated by the genus *Methylosinus* (Fig. 5c). Drought decreased the relative abundance of *Methylosinus* in all the heavy fractions of the fractionated DNA gradient (Fig. 5c). Besides *Methylosinus*, the other active microbial taxa have been given in Fig. S3.

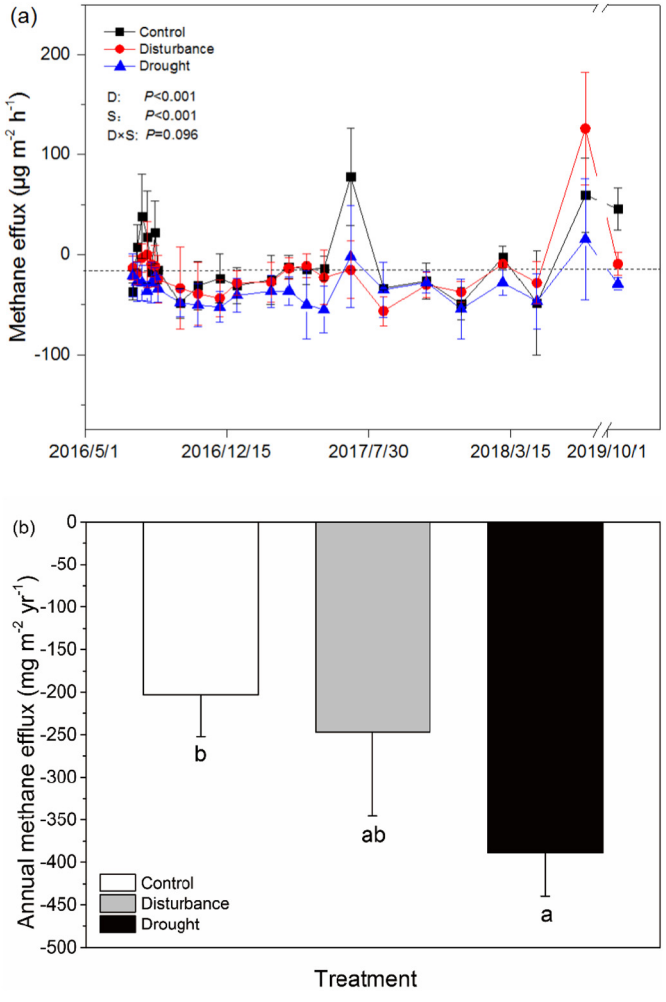

Fig. 3. Variations in (a) soil *in situ* CH_4 efflux between July 2016 and October 2019 and (b) annual CH_4 efflux between July 2016 and October 2019 in a subtropical evergreen forest. Values are means ± standard errors. Negative values indicate CH_4 uptake. D, drought treatment; S, sampling time. Different lowercase letters indicate significant differences.

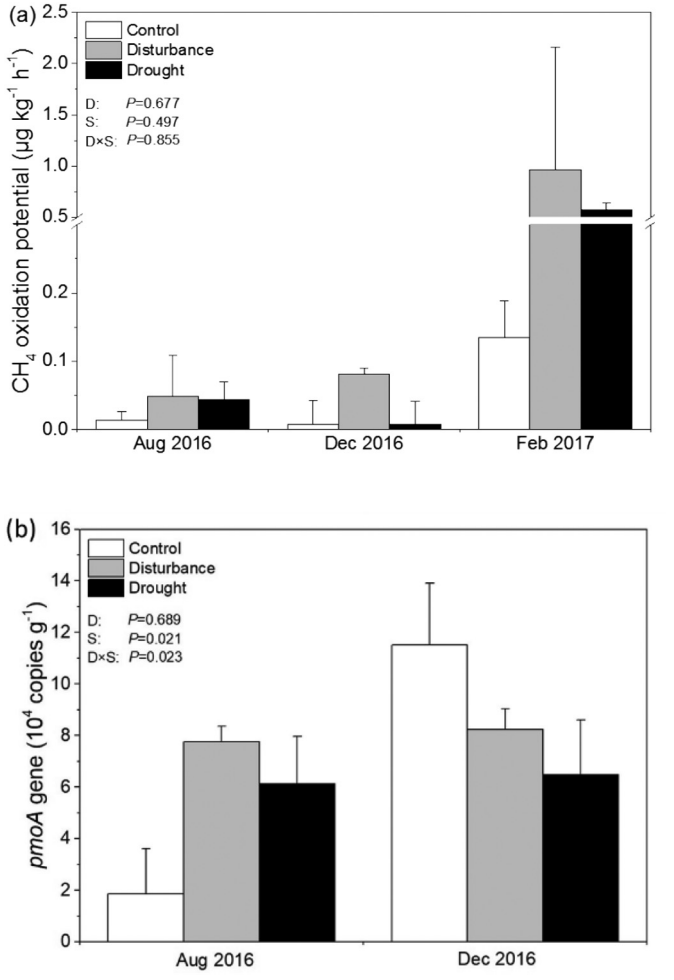

Fig. 4. Changes in (a) soil CH_4 oxidation potential under the ambient atmospheric CH_4 concentration and (b) *pmoA* gene copy numbers in soils of a subtropical evergreen forest between August 2016 and February 2017. Values are means ± standard errors. D, drought treatment; S, sampling time.

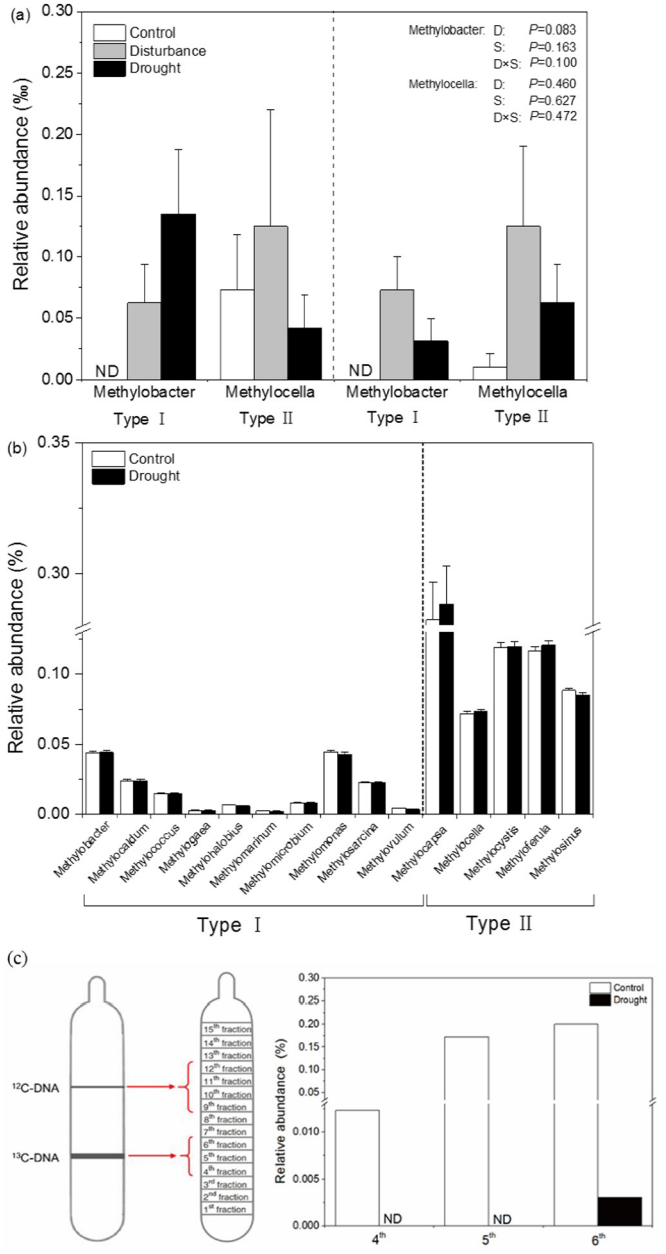

Fig. 5. Relative abundance of methanotrophs in soils of a subtropical evergreen forest sampled in August 2016 (left) and December 2016 (right) based on *16S* rRNA high-throughput sequencing (a), metagenomic sequencing (b) and the most abundant methanotrophic genus (*Methylosinus*) of fourth, fifth and sixth fractions of DNA based on $^{13}CH_4$ labelling in combination with *16S* rRNA high-throughput sequencing (c) from soils sampled in December 2016. Values are means ± standard errors. D, drought treatment; S, sampling time; ND, not detected.

3.4. The key factors driving soil CH_4 uptake across forest biomes

We used structural equation modeling to quantify the relative contributions of the direct and indirect drought-derived pathways to soil CH_4 uptake. First, we quantified the key factors driving soil CH_4 uptake and found that the SEM model could explain 81% of the variation in soil CH_4 uptake in the subtropical forest (Fig. 6). According to this model analysis, we found that the direct pathway (*i.e.*, soil aeration) could can explain 36% of the variations in soil CH_4 uptake, whereas the indirect pathway (*i.e.*, community structure and abundance of methanotrophs) could explain 27% of the variation. It is interesting to note that methanotrophic oxidation potential could explain for 27% of the variation in soil CH_4 uptake but methanotrophic oxidation potential is not directly influenced by soil aeration under drought (Fig. S4). Secondly, we would like to extrapolate these relationships between soil moisture content, methanotrophic communities and soil CH_4 uptake across forest biomes. We have to acknowledge that there are no data available on the methanotrophic communities under drought across forest biomes, according to our meta-analysis literature (Table S1). However, we found that there are similar correlations between soil moisture contents and CH_4 uptake in the subtropical forest ($r^2 = 0.16$, $P < 0.01$, Fig. S5a) and across forest biomes (Fig. S6), whereas soil temperature had only a very weak influence on soil CH_4 uptake in the subtropical forest ($r^2 = 0.02$, $P < 0.01$, Fig. S5b).

4. Discussion

Drought can greatly influence soil functions, including soil CH_4 uptake in forest ecosystems (Wang et al., 2012; Anderegg et al., 2013; Schlesinger et al., 2016). By combining a meta-analysis and measurements of 3-year *in situ* CH_4 uptake, we can provide clear evidence that drought influences soil CH_4 uptake *via* direct and indirect pathways across forest biomes. In general, in well-aerated forest soils, CH_4 production rates are negligible, so CH_4 uptake can be used to represent CH_4 efflux, as the latter is the net effect of the processes of CH_4 production and CH_4 uptake (Bu et al., 2019; Gu et al., 2019; Nazaries et al., 2011; Kolb, 2009; Tate, 2015; Zhou et al., 2021). However, we acknowledge that after heavy rain, especially in summer, there were several occasions of CH_4 emission (Fig. 2), indicating that CH_4 production was higher than CH_4 uptake. Moreover, our results highlight the importance of methanotrophic communities in explaining the variance in soil CH_4 uptake in forest ecosystems.

5. Effects of drought on soil CH_4 uptake across forest biomes

Forest ecosystems act as the largest CH_4 sink in terrestrial ecosystems (Dutaur and Verchot, 2007). Most previous studies have focused on the effects of drought on soil CH_4 uptake in tropical and temperate forests (Table S1). To our surprise, we did not find any studies on soil CH_4 uptake under drought in subtropical forests (Table S1). In fact, subtropical forests in China represent a specific forest biome, as most regions belong to mountains and deserts at a latitude of between 23° and 32° across the world (Bu et al., 2018). Previous studies have shown that subtropical forests in China can oxidize ~0.295 Tg CH_4 per year, accounting for 44% of the total CH_4 sink in forest soils across China and offsetting 10.9% of the annual CH_4 emissions from Chinese wetlands (Chen et al., 2013; Wang et al., 2014). In this study, we measured a mean *in situ* CH_4 uptake of 23.17 ± 5.59 μg m^{-2} h^{-1} (Fig. 3), which is lower than that of tropical forests (24.87 ± 18.79 μg m^{-2} h^{-1}) and temperate forests (73.50 ± 96.02 μg m^{-2} h^{-1}) on the basis of the meta-analysis (Table S1). We also noticed that soil CH_4 oxidation rates showed wide variation in the subtropical forests (Fig. 4), which was consistent with previous studies (Lu et al., 2019; Peichl et al., 2010; Tate, 2015; Ni and Groffman, 2018). We speculate that, except for several occasions of CH_4 emission, large variations in CH_4 uptake were attributed to CH_4 production rates. However, we found that there were similar CH_4 production rates among the treatments after the inhibition of CH_4 oxidation rates (Bu et al., 2019), which was confirmed by the similar soil extractable organic carbon and nitrogen contents, which acted as substrates for methanogenesis in the subtropical forests (Bu et al., 2018).

Given that soil CH_4-oxidizing bacteria are responsible for CH_4 uptake, methanotrophic activities respond to many soil properties such as texture, structure, temperature and nutrient contents, among which soil moisture content plays the predominant role in driving soil CH_4 uptake in forest ecosystems (Kolb, 2009; Zhou et al., 2014; Fest et al., 2015; Tate, 2015). Drought increases the diffusion rates of atmospheric CH_4, thus enhancing the availability of CH_4 to methanotrophs across the soil profile, supported by lower soil moisture contents

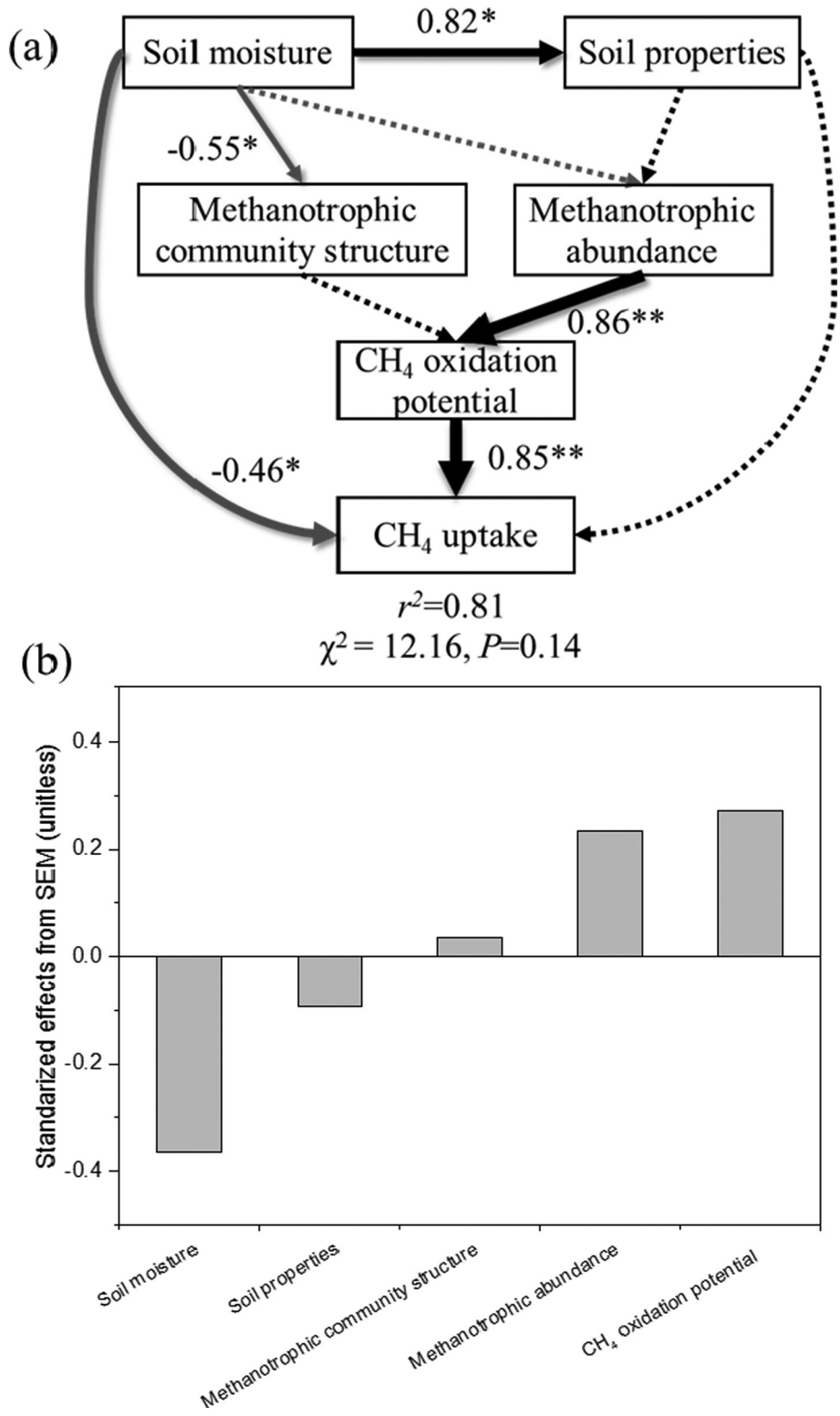

Fig. 6. Structural equation model describing the effects of soil moisture, soil properties (pH, soil total C and N contents, soil NH_4^+–N and NO_3^-–N contents), methanotrophic community and methanotrophic abundance on soil CH_4 oxidation potential as well as *in situ* soil CH_4 efflux in a subtropical forest (a). Arrow widths are proportional to the strength of the relationship. The dotted lines indicate no significance. Significance levels are as follows: *, $P < 0.05$; **, $P < 0.01$. The standardized total effects (direct plus indirect effects) derived from the structural equation model were also calculated (b).

under drought in these subtropical forests (Dumont et al., 2011). Given that there were no differences in soil CH_4 uptake between the disturbance treatment and the ambient control (Fig. 3a), we are sure that drought rather than shade effects played the major role in driving soil CH_4 uptake across forest ecosystems (Fig. 6).

Previous studies have demonstrated that soil temperature can also greatly influence soil CH_4 uptake (Kolb, 2009; Tate, 2015). In contrast, we did not find strong relationships between soil temperature and soil CH_4 uptake in these subtropical forests ($r^2 = 0.02$, $P < 0.05$; Fig. S5b). The reason for this could be attributed to minor variations in soil

temperature between the drought treatment and the ambient control (data not shown). By comparison, soil aeration overrode soil temperature to exert an effect on soil CH_4 uptake in the subtropical forest (Fig. S5a) and across forest ecosystems (Fig. S6).

6. Microbial mechanisms of soil CH_4 uptake under drought in forest ecosystems

Besides the direct pathway, drought can alter methanotrophic communities and methanotrophic activities (the indirect pathway) in forest ecosystems (Fig. 1). Many studies have demonstrated that drought can increase *in situ* soil CH_4 uptake in forest ecosystems (Tate, 2015; Liu et al., 2019; Feng et al., 2020). However, to our surprise, no studies have investigated changes in both *in situ* CH_4 uptake and the associated methanotrophic communities under drought across forest ecosystems (Table S1). To our knowledge, our work is the first study to investigate the effects of drought on *in situ* CH_4 efflux and the underlying microbial mechanisms.

We noticed that there were no marked differences in the abundance of the key functional genes involved in CH_4 oxidation (Fig. 4) and methanotrophic communities (Fig. 5) between the drought treatment and ambient control forests. Similar methanotroph abundance and community structure might contribute to similar methanotrophic activities, as previous studies have shown that methanotrophic activities are strongly linked to changes in methanotrophic communities (Kolb, 2009; Tate, 2015; Pratscher et al., 2018; Feng et al., 2020). On the other hand, a recent study has shown that the methanotrophic genus *Methylocapsa* is widespread and can oxidize atmospheric CH_4 (Tveit et al., 2019). We speculate that this specific genus of methanotrophs might be responsible for atmospheric CH_4 oxidation in this subtropical forest. However, we found that active methanotrophic communities were dominated by *Methylosinus* (see Fig. 5c). Our results indicated that *Methylosinus* rather than *Methylocapsa* mediated soil CH_4 oxidation in this forest ecosystem. Here, we provide further evidence of conventional methanotrophs being important CH_4 consumers under atmospheric CH_4 concentrations (Cai et al., 2016). In addition, we noted that we detected only aerobic methanotrophic communities but not the anaerobic CH_4–oxidizing microorganisms in this forest soil (data not shown) (Segarra et al., 2015).

Although the methanotrophic communities were similar, we found that methanotrophic communities played the second most important role after soil moisture contents in driving soil CH_4 uptake under drought in forest ecosystems (Fig. 6). We could use soil moisture contents alone to predict changes in soil CH_4 efflux across forest ecosystems (Fest et al., 2015; Liu et al., 2019). However, if we consider both soil moisture contents and methanotrophic communities, we can improve the efficiency of our predictions of the effects of drought on soil CH_4 uptake by 74% in forest ecosystems (Fig. 6).

7. Conclusions

From the findings of the meta-analysis and the 3-year *in situ* CH_4 uptake measurements to examine the effects of drought on soil CH_4 uptake across forest biomes, we conclude that drought has driven soil CH_4 uptake *via* direct and indirect pathways: the former enhances CH_4 oxidation by increasing soil aeration; the latter inhibits CH_4 oxidation through altering the methanotrophic community structure, but the influence of the direct pathway was greater. Considering that methanotrophic communities can greatly improve our SEM performance, we therefore suggest that methanotrophic communities should be incorporated into future Earth System models to improve our understanding of changes in *in situ* soil CH_4 uptake across forest biomes. Comprehensive investigations of *in situ* soil CH_4 efflux and the associated methanotrophic communities are needed for future research into terrestrial ecosystems.

The schematic graph shows variations in soil CH_4 uptake under drought in a subtropical evergreen forest. Drought significantly increased soil CH_4 uptake by increasing soil aeration without changing the oxidation potential and abundance of methanotrophs. Similar methanotrophic communities were also observed in the drought and ambient control plots according to metagenomic sequencing analyses. Active methanotrophic communities were dominated by Type II methanotrophs, as found by DNA stable-isotope probing analysis.

CRediT authorship contribution statement

Xiaoqi Zhou: Conceptualization, Investigation, Resources, Software, Writing – original draft, Visualization, Validation. **Mingyue Zhang:** Investigation, Writing – original draft, Visualization, Validation. **Sascha M.B. Krause:** Writing – review & editing, Visualization, Validation. **Xuelei Bu:** Investigation, Visualization, Validation. **Xinyun Gu:** Visualization, Validation. **Zhiying Guo:** Visualization, Validation. **Zhongjun Jia:** Visualization, Validation. **Xuhui Zhou:** Visualization, Validation. **Xihua Wang:** Visualization, Validation. **Xiaoyong Chen:** Visualization, Validation. **Yanfen Wang:** Resources, Software, Visualization, Validation.

Declaration of competing interest

The authors declare no competing interests.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (No. 31870497, No. 31600406 and No. 31630008), the Shanghai Science and Technology Innovation Fund (No. 18391902300), the Fundamental Research Funds for the Central Universities and the East China Normal University Multifunctional Platform for Innovation (008).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.148292.

Further Reading

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