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Enhanced soil methane oxidation in both organic layer and topsoil during the succession of subtropical forests

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ABSTRACT

Although (sub)tropical forests account for 10–20% of the atmospheric methane (CH_4) uptake by soils, the study of soil CH_4 oxidation rates and the controlling factors during the chronosequences of forest succession remains poorly understood. The objectives of this study were to characterize the vertical distribution patterns and dynamics of CH_4 oxidation among the early-, mid-, and late-successional stages of subtropical forests, and to investigate the main drivers of soil CH_4 fluxes. Three successional forests soils were collected and the ambient and potential CH_4 oxidation rates, the related enzymes as well as the key soil parameters were determined in the laboratory. The soils at mid- and late-successional stages functioned exclusively as a CH_4 sink while the soil at early-succesional stage was either a CH_4 source or sink. Soil CH_4 oxidations showed significant vertical distributions along with the successional gradients. The highest rate of ambient CH_4 oxidation was observed in the A-horizon of the mid-successional stage (forest age $\sim$ 100 years), increased by 26-fold compared to the early successional stage, while the highest rate of potential CH_4 oxidation was detected in the O-horizon of the late-successional stages (forest age > 300 years), which increased the CH_4 oxidation by 29% and 21% respectively compared to the early- and mid-successional stages. Soil CH_4 oxidation enhanced with decreasing of soil nitrite and nitrate content but weakened with declining of soil moisture at the successional chronosequence. Collectively, subtropical forests have the potential to increase the soil sink capacity for CH_4 oxidation along the successional gradient, and thereby providing the negative feedbacks to ecosystems under climate changing.

1. Introduction

On the 100-year timescale, the global warming potential of methane (CH_4) is 25 times that of carbon dioxide (CO_2), and CH_4 is the second important greenhouse gas (GHG) in terms of its contribution to climate change, accounting for approximately 20% of the global GHG effect (IPCC, 2013). Over the past 150 years, the methane concentration in atmosphere has increased by 150% (Montzka et al., 2011). The rate of increase in atmospheric methane concentrations is determined by the difference between methane sources and sinks, and currently the terrestrial ecosystem is a net source of atmospheric methane (Tian et al., 2016).

It is well known that well-aerated forest soils are globally important carbon sinks that can mitigate atmospheric methane concentration increase (Basiliko et al., 2009; Chang et al., 2021; Howden et al., 2008). The annual uptake of methane indicating the consumption processes of CH_4 oxidation at both aerobic and anaerobic conditions by global soils ranges from 26 ~ 36 Tg, of which soils account for approximately 10% (Christiansen et al., 2016), and deciduous forests are found to be the strongest sinks of atmospheric CH_4 (Degelmann et al., 2009; Liu and Greaver, 2009; Mazza et al., 2021). Globally, primary forests are at risk of destruction due to human factors (deforestation, man-made fires) and natural factors (such as drought and natural fires). The responses of forests at different succession stages to global changes, such as the
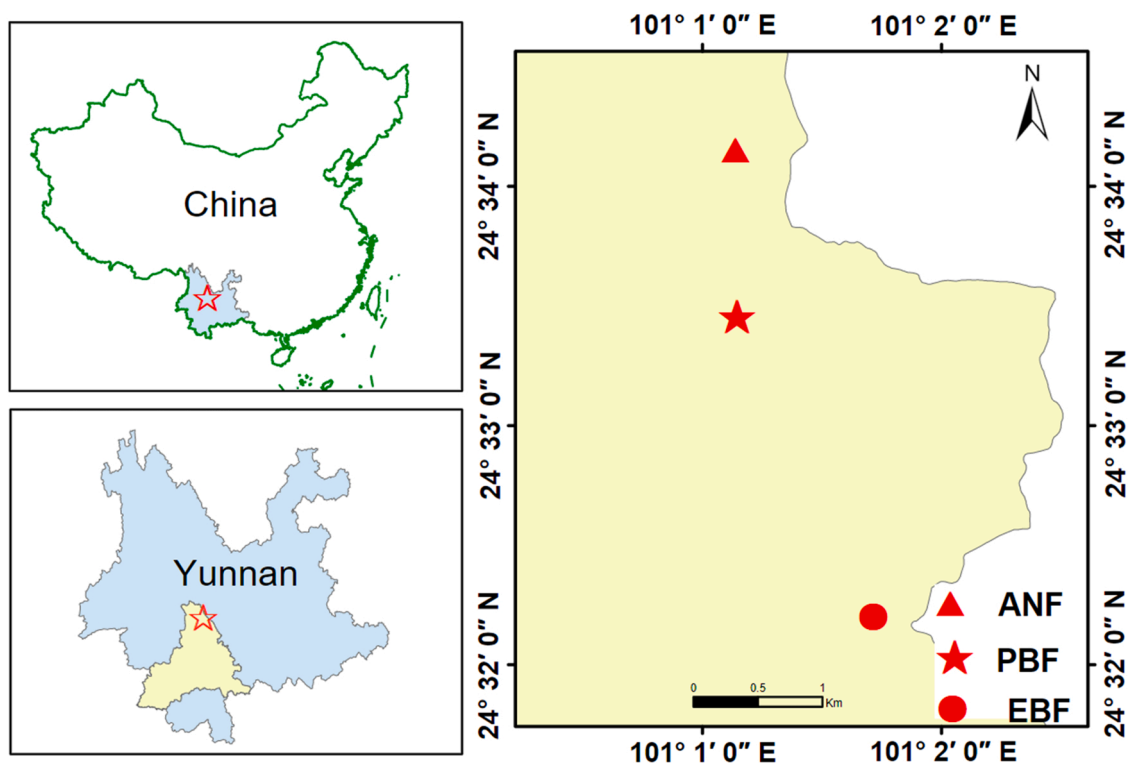

Fig. 1. Location map of the study sites.

oxidation and emission of methane, are still unclear (Tate, 2015). As an important soil biochemical process, methane oxidation is based on the principle that methane is transported to the aerobic area of the soil through the soil pores, and methane oxidizing bacteria can oxidize the surrounding methane into carbon dioxide, thereby effectively reducing methane emissions and alleviating the greenhouse effect (Le Mer and Roger, 2001; Dutaur and Verchot, 2007),.

China's forest area is 208 million ha, accounting for 22% of the national area and it contains large methane oxidation potential. The annual uptake of methane by forest soil in China contributed 3.6% of the global methane sink (Cai, 2012), thus the dynamic change of methane oxidation by forest soil is one of the key factors affecting the methane concentration in the atmosphere (Basiliko et al., 2009; Wei et al., 2018). Changes in forest soil CH_4 production and oxidation are affected by various factors such as soil moisture (Sihi et al., 2021; Subke et al., 2003; Wang et al., 2013) and soil temperature (Grogan et al., 2004; von Arnold et al., 2005; Zhu et al., 2021) as well as the soil N availability via soil N transformation and plant uptake (Fender et al., 2012; Ren et al., 2022). Soil moisture is a key driver of CH_4 oxidation rates from forest soil because the water-filled porosity limits the transport and diffusion of the oxygen (O_2) and CH_4 in soils (Song et al., 2008; Xiang et al., 2022). As for the temperature effects on CH_4 fluxes, results were inconsistent and tends to be co-affected by the dynamic changes of soil dissolved organic carbon and inorganic nitrogen (Rask et al., 2002; Borken et al., 2006; Liu et al., 2007; Chen et al., 2011; Mills et al., 2013; Li et al., 2021).

Subtropical evergreen broad-leaved forest is the forest with the widest distribution, the largest area and the highest biodiversity in China, accounting for about 1/4 of China's land area (Chen et al., 2019), and this high biodiversity in the subtropics makes it challenging to explore how changes in nutrient status and availability along the forest successional gradients affect the biogeochemical cycling, such as the greenhouse gas emission and uptake (Liu et al., 2020). On both regional and global scale, the rates of primary forest loss and secondary forest generation are increasing in the (sub)tropics (Hansen et al., 2013; Sullivan et al., 2019), creating a requirement for the quantitative assessment of how methane oxidation patterns respond to secondary

succession, and which soil physiochemical factors as well as biotic factors controlling the methane emissions. A recent global meta-analysis showed that primary forest conversion significantly increases soil CH_4 emissions (Han and Zhu, 2020). The methane fluxes from forest soils in successional chronosequences may quite different, due to the shifts in the plant communities, and the age of second forest and the human disturbance and management (Hergoualc'h and Verchot, 2011; Kim and Kirschbaum, 2015), however, the characteristics of methane fluxes or uptake during subtropical forest succession are still unknown.

Similar to other biogeochemical processes, there are vertical distributions in the ability of soils to oxidize CH_4 and this depths difference may affect the CH_4 uptake capacity by soil profiles and is a critical parameter for the CH_4 emission models. Some studies have shown that the highest value of CH_4 oxidation activity in temperate forest soil was located in the subsurface soil (Koschorreck and Conrad, 1993; Plain and Epron, 2021) while other findings illustrated the highest CH_4 oxidation rates was in the surface/topsoil soil (Borken et al., 2003; Sjogersten et al., 2007; Takahashi et al., 2021). For example, Borken et al. (2003) found that the vertical distribution of CH_4 oxidation rates in forest soil varied with the tree species: the highest rate of CH_4 oxidation was located in the subsurface soil of coniferous forest (with spruce and pine as dominant species), but in a broad-leaved forest soil (with beech as the dominant species) the highest value was located in the topsoil. The study by Sjogersten et al. (2007) also showed that the highest methanotrophic activity at a mountain birch forest-tundra system occurred in topsoil. It can be seen that the vertical distribution of CH_4 oxidation in forest soils may vary with climate, biological, soil and other factors. Over the short distances within a subtropical forest, soil abiotic and biotic factors rather than meteorological factors, might control the substantial variations in nutrient supply and the processes of CH_4 production-oxidation. However, compared with the studies of soil methane emissions in boreal and temperate forests, observations for soil methane oxidation in (sub) tropical forests, especially for the forest succession stands and the vertical distribution patterns are still sparse and elusive, implying high uncertainty in estimates of terrestrial ecosystem CH_4 fluxes.

To address the gap of methane oxidations in subtropical forest soils,

including the vertical distribution patterns of the methane oxidation capacity and dynamics during the forest succession, we adopted the method of "space instead of time" and selected three typical succession stages in a national Natural Reserve: the early-successional stage of *Alnus nepalensis* forest (ANF) dominated by *Alnus nepalensis* with the age around 60 years; the mid-successional stage of *Dianshan poplar* forest (PBF) dominated by *Populus bonatii* with the age around 100 years; and late-successional stage of the evergreen broad-leaved forest (EBF) with the age > 300 years and has been well protected from human disturbance (Li et al., 2016). The early- and mid-successional forests developed through natural succession with the gradual invasion of some pioneer broadleaf species. They are all located in the Ailao Mountains, southwest Yunnan Province, China. The objectives of this study were to (i) explore the effects of forest succession on soil CH_4 oxidation in subtropical areas, including the ambient and potential oxidation capacity; (ii) further characterize the forest succession effects in different soil layers; (iii) reveal the main controlling factors for soil CH_4 oxidation during the forest succession. We hypothesized that the primary forest has the highest capacity for CH_4 oxidation due to the increased activity of methane monooxygenase induced by the increased soil organic carbon accumulation, and the CH_4 oxidation capacity faded with the soil depth extending due to the more air-filled pore space resulting in greater CH_4 diffusing into the O-horizon.

2. Materials and methods

2.1. Study area

This experiment field is located in the Ailaoshan Station of Subtropical Forest which belongs to the Chinese Academy of Sciences, and it is also part of the Ailaoshan Nature Reserve (23°35′–24°44′ N, 100°54′–101°30′ E), which is located in Jingdong County, Yunnan Province, southwest China (Fig. 1). The soils of the three successional forests belong to the Yellow-brown soil and all located in the Nature Reserve with no human disturbance and no cultivation. This area is in a subtropical mountain climate which belongs to the monsoon climate zone. The altitude is 2300 ~ 2600 m and the annual average temperature was 11.3 °C and the average temperature of the growing season was 15.7 °C. The annual precipitation was 1947 mm, of which 85% of the precipitation occurred in the rainy season (from May to October). The three representative succession forests were selected and the detailed information of them was as following:

Alnus nepalensis forest (ANF) is a secondary forest after the deforestation and burning of the evergreen broad-leaved forest, which is located in the northwest corner of the Ailao Mountain Ecological Station, with an area of about 100 hm². It is in the early-successional stage and the recovery period is about 60 years. The ANF is considered to be the primary vegetation/stage after disturbance. The tree layer is composed of a single tree species of *Alnus nepalensis*, and the main species of the shrub layer are *Rhododendron delavayi*, *Lyonia ovalifolia* and *Dchroafebr fuga*. This type of forest canopy is sparse, with the canopy closure of around 75%, and the tree height is around 15 m (Li et al., 2016).

Populus bonatii forest (PBF) is also a secondary forest and formed after the deforestation and burning of the evergreen broad-leaved forest and it is a pioneer tree species for plant community succession in this region. The recovery period of PBF is about 100 years and it is in the mid-successional stage. The canopy closure is 80–85% and the tree height is 15–20 m. The PBF area are scattered as small patches in the primary forest of Evergreen broad-1eaved forest (PBF). The dominant species in the tree layer are aspen poplar, accompanied by various Fagaceae plants such as *L. hancei*, *Ternstroemia gymnanthera*, and some species of shrubs and herbaceous vegetation (Li et al., 2016).

Evergreen broad-1eaved forest (EBF) is more than 300 years old and is the late-successional stage. It is the largest and most complete native vegetation in the Ailao Mountain, accounting for about 78% of the total vegetation area. The vertical stratification of the community is clear, the

tree layer height is 20–25 m, and the canopy closure is 95%. The dominant species are *Lithocarpus xylocarpus*, *L. crassifolius*, *Castanopsis rufescens*, and *Schima noronhae* (Li et al., 2016).

2.2. Experimental design

In each succession forest, eight plots with 5 m × 5 m at least 10 m distances between each other were established randomly as spatial replicates at the field sites (together there were 24 plots in the three succession forest). The layers of soils used in the experiment were collected in 2021 April. The details of the two layer soils were as following: one is the top O horizon with loose and partly decayed organic matter (approximately 5–8 cm, 6–10 cm and 15–20 cm for ANF, PBF and EBF respectively), the other is the A horizon with organic-rich mineral soil of ~10 cm depth below the O horizon. The two layer soils were sampled from the above mentioned eight replicate plots of each succession forest in field sites, respectively. The five-point sampling method was used to collect 5 soil cores by a steel corer from each plot and was mixed by hand to remove the coarse roots a portion of soil was removed from each sample and packed into a 2 mL centrifuge tube, then stored in a − 80 °C freezer for enzyme activity and microorganisms, and the remaining soil was stored at − 4 °C. Before the experiment started, 25 g of soil was added to the 250 mL flask and then the air in the flask was flushed, followed with the flask was capped. Culture experiments were performed at the temperature of 15.7 °C, which was based on the average temperature of the growing season. In order to maintain the soil water content as in situ, we did not artificially adjust the water content, but only weighed and replenished water every day during the incubation to keep the water content consistent with the initial value of sampling.

Two culture experiments with different initial methane concentration were performed separately: a) monitoring the methane oxidation rates of the three succession forest soils at the ambient methane concentration (approx. 1.85 ppm). The atmospheric methane is mainly consumed by a group of methane-oxidizing bacteria (MOB) that using atmospheric methane as their main energy source and sensitive to low concentration of methane (by high affinity methanotrophs). During the culture process, no exogenous methane was added to the flask and the methane oxidation rates were measured once a day under the atmospheric concentration for three days. After each gas sampling, the flask was blow with a fan for 20 min to fully exchange with the air outside. The flask cap was opened and sieved with a Parafilm (air ventilate but no water escape) after each measurement to maintain the methane status as the ambient condition and to avoid moisture loss; b) monitoring the methane oxidation potentials at the high methane concentration in the surroundings (approx. 1000 ppm CH_4, by low affinity methanotrophs), and the potential assays offer two unique advantages. First, it was capable of measuring the maximum functional capacity of MOB communities because the differences between the successions/sites in potential methane oxidation should reflect changes in MOB community or enzyme activity (Sullivan et al., 2013). Second, potential assays could minimize other important constraints that obscure biogeochemical process, i.e., there are anaerobic micro-zones in the topsoil, and 80% of the endogenous high-concentration methane (18–1000 ppm) produced in the anaerobic zone could be consumed by the low-affinity MOB before escaping into the atmosphere (Cai et al., 2016). Therefore, we observed the ambient and potential CH_4 oxidation rates using the methods described by Cai et al. (2016). For the measurement of methane oxidation potentials, the high-concentration methane was injected into the flasks and the methane concentration was adjusted to approximately 1000 ppm as the reference used (Cai et al., 2016). The soil was pre-cultured at 15.7 °C for 3 days to stabilize soil microbial activity, then the air in the bottle was flushed again, and the methane concentration in the bottle was adjusted to 1000 ppm again to start the measurement. The methane concentration in the flask space was measured every 24 h for 10 days and the cap was not opened, because of the two reasons, on one hand, it is necessary to continuously measure the change of methane
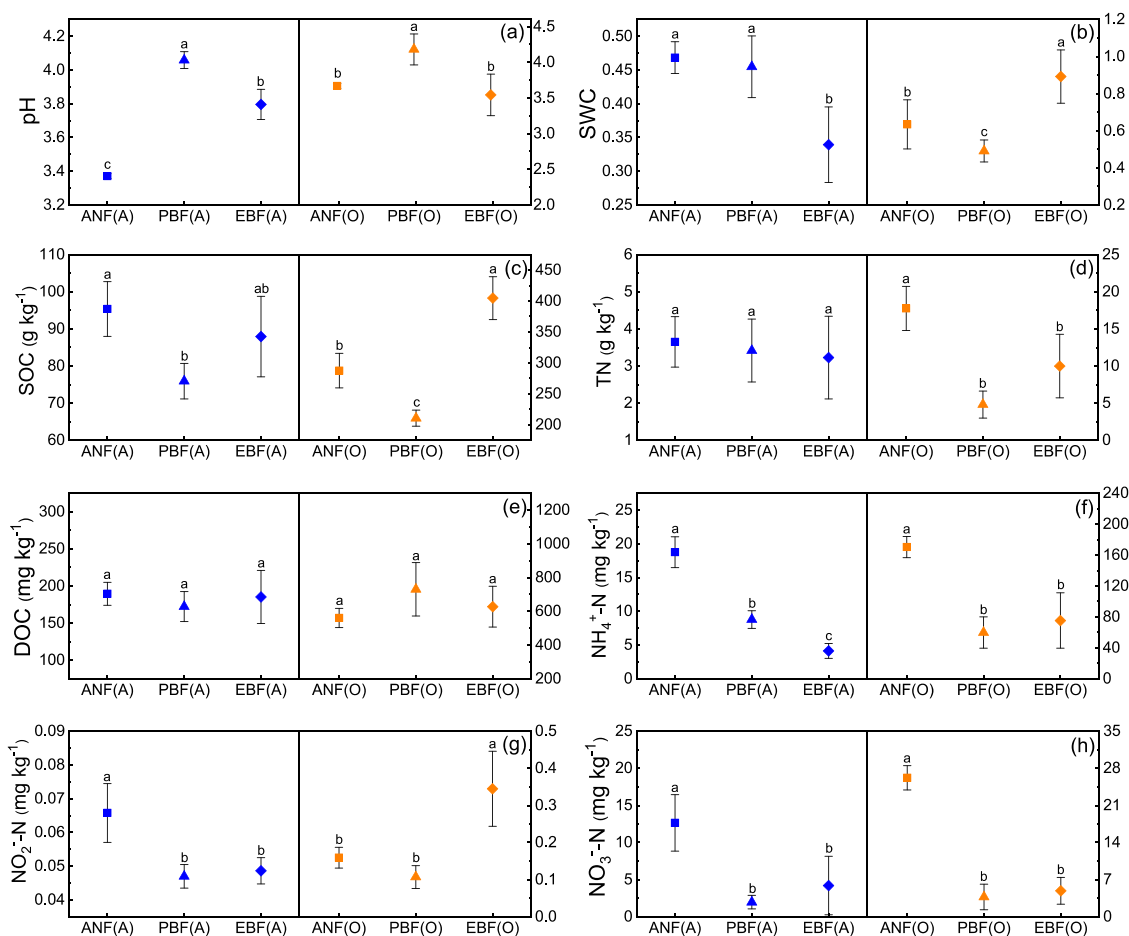
Fig. 2. Soil physicochemical properties in three forest succession stages. Note: Different lowercase letters denote significant differences between the three forests ($P < 0.05$). The blue color represents A-horizon and the orange color represents O-horizon. SWC: soil water content; SOC: soil organic carbon; TN: soil total nitrogen; DOC: dissolved organic carbon. ANF: *Alnus nepalensis* forest (early-stage); PBF: Populus bonatii forest (mid-stage), EBF: Evergreen broad-leaved forest (late-stage).

concentration, and on the other hand it is to prevent soil moisture from escaping. For both of the two culture experiment, a 10 mL sample was collected from the headspace by using a 10 mL syringe, then 10 mL of synthetic air (80% N_2, 20% O_2) was injected into the flask to maintain the air pressure in the bottle. The concentration of methane was measured by a gas chromatograph (Shimadzu, Japan, GC-2014).

2.3. Analysis of soil physicochemical properties

The soil pH was measured via a pH-meter under the condition of soil to water ratio of 1:2.5. The nitrate (NO_3^--N), nitrite (NO_2^--N), ammonium (NH_4^+-N) and dissolved organic carbon (DOC) were extracted with 1 mol L^{-1} KCl solution, and their concentrations in extracts were determined by spectrophotometer method in an Intermittent Chemical Analysis (CleverChem380; DeChem-Tech; Germany). Soil samples were digested by sulfuric acid and then the total nitrogen (TN) was determined by the sodium salicylate method. Soil organic carbon (SOC) was measured by converting total organic carbon into CO_2 through high temperature combustion and catalytic oxidation with an organic carbon analyzer (VarioTOC ; Elementar, Germany).

2.4. The CH4 emission rate

Methane emission rate was calculated with the following formula:

$$F = \frac{M}{V_m} * (VC_2 - VC_1) * \frac{1}{1000m} * \frac{P}{P_0} * \frac{T_0}{T}$$

Where: F is the CH_4 emission (ng (CH_4)•kg^{-1}soil•d^{-1}); M is the molar mass of CH_4 (16 g•mol^{-1}); V_m is the molar volume of 22.4 (L•mol^{-1}); V is the headspace volume (mL); C_1 is the concentration of the gas to be determined (ppm) in the container; C_2 is the concentration of the gas to be determined (ppm) in the container after one day incubation day; m is the mass of dry soil (g); P is 80.735 kpa and P_0 is 101.325 kpa, which represent the atmospheric pressure in Kunming, China (experimental area)and the standard atmospheric pressure, respectively; T_0 is 273.15 K and T is (273.15 +t (℃)) K, which represent the absolute temperature and the temperature controlled in this experiment under the standard conditions, respectively. Detailed information can be found in Yang et al., 2022.

The cumulative CH_4 emission:

$$E = \sum_{i=1}^{n} \frac{F_i + F_{i+1}}{2} \times (t_{i+1} - t_i)$$

E is the cumulative flux of CH_4 (ng CH_4 Kg^{-1}), F is the instantaneous flux rate of CH_4 (ng CH_4 Kg^{-1} d^{-1}), i is the ith measurement, and (t_{i+1} −ti) is the time interval between the two measurements (X. Yang et al., 2022; Z. H. Yang et al., 2022).

2.5. Analysis of enzyme activities

The soil methane monooxygenase (MMO) activities were measured by a colormetric plate assay, and the samples for MMO measurements were from the incubation experiments. In brief, soil slurries (soil: solution = 1:100) were buffered with the 50 mM sodium acetate (pH =4.5),

Table 1

Means of the determined parameters for the A- and O- horizons in different forest succession stages. ANF: *Alnus nepalensis* forest (early-stage); PBF: Populus bonatii forest (mid-stage), EBF: Evergreen broad-leaved forest (late-stage), with standard error of the mean. Significant soil depth effects at a given forest are indicated by different indices (a, b). Note that forest succession effects are not illustrated in this table, they are showed in Fig. 1.

	ANF		PBF		EBF	
	A-horizon	O-horizon	A-horizon	O-horizon	A-horizon	O-horizon
CH_4 cumulation (ng kg^{-1} dry soil)	-2.92 ± 0.07 a	-2.56 ± 0.52 a	-3.57 ± 0.20 b	-2.49 ± 0.14 a	-3.15 ± 0.17 a	-4.42 ± 0.43 b
pH	3.37 ± 0.01 b	3.67 ± 0.02 a	4.06 ± 0.03 a	4.18 ± 0.14 a	3.80 ± 0.06 a	3.54 ± 0.19 a
SWC (w/w)	46.84 ± 1.57 a	63.44 ± 8.80 a	45.48 ± 3.05 a	49.10 ± 3.94 a	33.94 ± 3.73 b	89.20 ± 9.56 a
NH_4^+-N (mg kg^{-1})	18.76 ± 1.53 b	170.34 ± 9.16 a	8.77 ± 0.87 b	59.76 ± 13.53 a	4.12 ± 0.73 b	75.31 ± 23.88 a
NO_3-N (mg kg^{-1})	12.65 ± 2.54 b	26.21 ± 1.53 a	1.97 ± 0.61 a	3.74 ± 1.61 a	4.21 ± 1.92 a	4.91 ± 1.68 a
NO_2-N (mg kg^{-1})	0.066 ± 0.001 b	0.16 ± 0.02 a	0.046 ± 0.002 b	0.11 ± 0.02 a	0.049 ± 0.003 b	0.34 ± 0.07 a
TN (g kg^{-1})	3.65 ± 0.45 b	17.76 ± 1.98 a	3.41 ± 0.56 a	4.81 ± 1.05 a	3.23 ± 0.74 b	9.99 ± 2.47 a
DOC (mg kg^{-1})	189.48 ± 10.28 b	560.43 ± 38.13 a	172.19 ± 13.45 b	730.27 ± 105.39 a	185.26 ± 23.85 b	627.35 ± 80.84 a
SOC (g kg^{-1})	95.36 ± 4.92 b	287.42 ± 18.62 a	75.92 ± 3.20 b	210.71 ± 8.66 a	87.92 ± 7.23 b	404.66 ± 23.11 a

which is close to the mean value of soil pH. All sample microplates were incubated in the dark at 25 °C for 3 h to determine the enzymes. After incubation, we used a 96-well microplate reader to determine the quantity of fluorescence for the enzymes, and the detailed procedure was shown in a previous study (Graham et al., 1992).

2.6. Statistical analysis

The data in this study were firstly analyzed for normal distribution by using the Kolmorov-Smirnov test, and then the variance homogeneity was checked by using the Levene test. One-way ANOVA and Fishers Least Significant Difference (LSD) were used to explore the significant differences of forest succession on each parameter of CH₄ oxidation, soil physicochemical properties and MMO activities. The interacting effects of succession stage and time (incubation date) were examined by using the multi-factor variance analysis. The potential correlations between the CH₄ oxidation and soil physicochemical properties and MMO activities were determined by Pearson correlation analysis. These statistical analysis was conducted by using SPSS (version 19, SPSS Inc., Chicago, USA), and data processing and drawing was used by Origin (version 2021, Origin Lab Inc., Northampton, USA).

3. Results

3.1. Soil physicochemical properties

Soil properties differed significantly among forest succession stages (Fig. 2) and A-horizons (Table 1). The differences of soil organic carbon (SOC), total nitrogen (TN), NH_4^+, NO_3 and pH existed across the three successional stages, and the effects of forest succession and A-horizons on soil pH, SOC and NH_4^+ were most pronounced (Fig. 2). The SOC content was lower in the early-stage of *Alnus nepalensis* forest (ANF) and mid-stage of Populus bonatii forest (PBF) relative to the late-stage of evergreen broad-1eaved forest (EBF) at O-horizon (Fig. 2c), indicating that with the developing process from secondary forest to the primary forest, soil carbon content tends to increasing. In contrast, the EBF induced pronounced decrease of NH_4^+ and NO_3 content in both the O- and A-horizons compared to the early-successional stage of ANF (Figs. 2f, 2h).

The contents of NH_4^+, NO_3, NO_2, SOC, TN and DOC in the O-horizon were significantly higher than that of the A-horizon soil, and there was a consistent trend in the three successional forests (Table 1). Soil pH was not different between the two layers across all plots at PBF and EBF forests and was fairly consistent acid (ranging from 3.54 to 4.18,
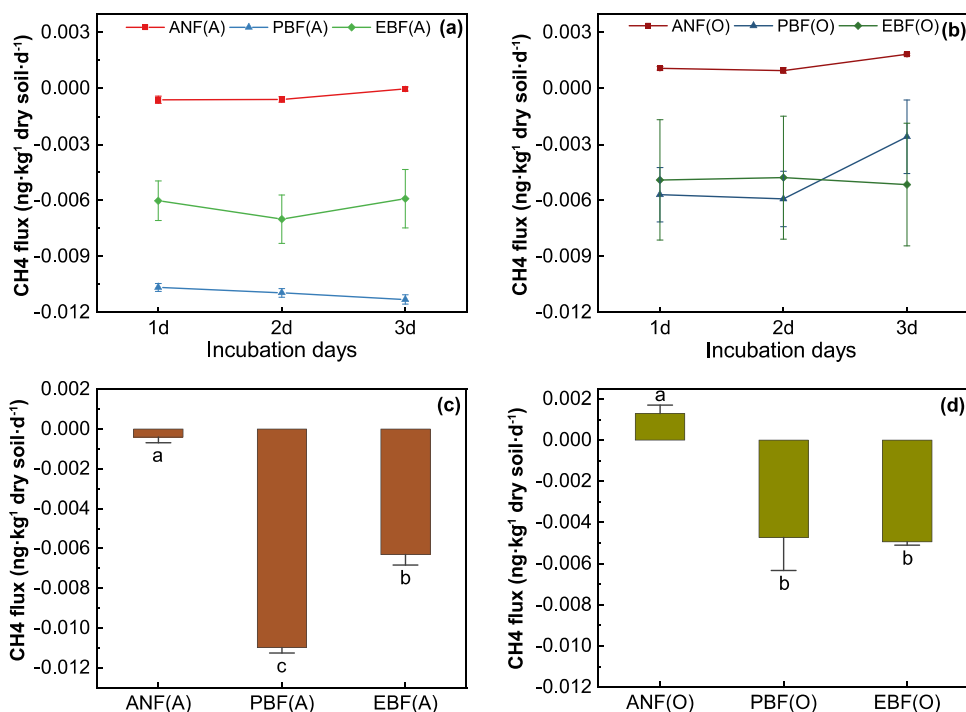

Fig. 3. Effects of forest succession on the rates of ambient CH₄ oxidation (the original CH₄ concentration of 1.85 ppm). Data are standard error ± mean (n = 8). The dynamic changes of the ambient CH₄ oxidation rates in A-horizon (Fig. 3a) and O-horizon (Fig. 3b), and the average rates of CH₄ oxidation in A-horizon (Fig. 3c) and O-horizon (Fig. 3d). Different lowercase letters denote significant differences between the three forests ($P < 0.05$). ANF: *Alnus nepalensis* forest (early-stage); PBF: Populus bonatii forest (mid-stage), EBF: Evergreen broad-leaved forest (late-stage).
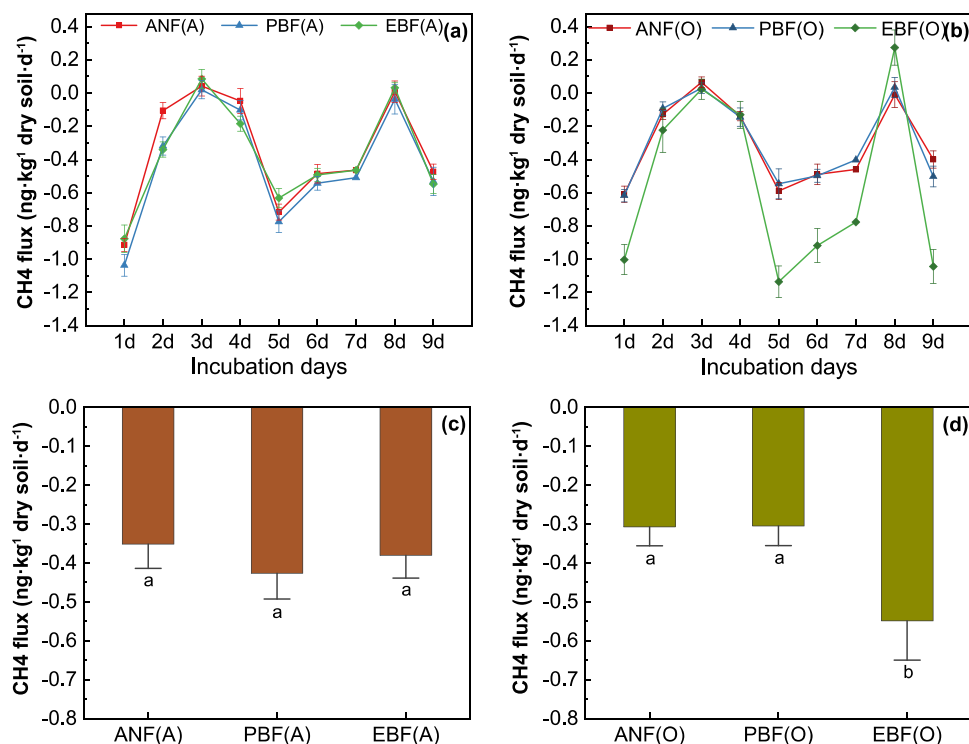
Fig. 4. Effects of forest succession on the rates of potential CH_4 oxidation (the original CH_4 concentration of 1000 ppm). Data are standard error ± mean (n = 8). The dynamic changes of the potential CH_4 oxidation rates in A-horizon (Fig. 4a) and O-horizon (Fig. 4b), and the average rates of CH_4 oxidation in A-horizon (Fig. 4c) and O-horizon (Fig. 4d). Different lowercase letters denote significant differences between the three forests ($P < 0.05$). ANF: *Alnus nepalensis* forest (early-stage); PBF: Populus bonatii forest (mid-stage), EBF: Evergreen broad-leaved forest (late-stage).

Table 2

Results of ANOVA testing the effects of the forest succession, incubation time and their interactions on the quantified parameters. Significant effects are indicated in bold.

	Forest succession (ANF vs. PBF vs. EBF)		Time		Forest succession *Time	
	F value	*P*	*F* value	*P*	*F* value	*P*
CH_4 flux in A-horizon	4.024	**0.019**	103.639	**< 0.001**	1.026	0.431
CH_4 flux in O-horizon	34.577	**< 0.001**	70.064	**< 0.001**	5.643	**< 0.001**

Table 1).

3.2. Effects of forest succession on the CH4 fluxes rates

During the incubation with ambient CH_4 concentration of approx. 1.84 ppm, the methane oxidation rates were monitored in O- and A-horizon soils for the three successional forests. In general, the mid- and late- stages of forests showed significantly stronger CH_4 oxidation than the early stage, and this pattern was found in both O- and A- horizons (Fig. 3). The mean daily rates of CH_4 fluxes in O-horizon were 1.29 ± 0.27, − 4.73 ± 1.07 and − 4.94 ± 0.11 ng kg^{-1}, and in A-horizon were − 0.41 ± 0.19, − 10.97 ± 0.19 and − 6.31 ± 0.35 ng kg^{-1} for early-, mid-, and late-stages respectively.

During the incubation with high CH_4 concentration of approx. 1000 ppm, the potential rates of methane oxidation were monitored for 9 days, and the CH_4 oxidation rates were about 100 times higher than the rates for ambient CH_4 oxidation (Fig. 4)., The mean daily rates of CH_4 fluxes in A-horizon were − 0.351 ± 0.042, − 0.426 ± 0.044 and − 0.380 ± 0.039 ng kg^{-1} for early-, mid-, and late-stage respectively (Fig. 4c) and there was no significant difference among the three successional forests (Fig. 4c), with the exception that the ANF showed significantly lower CH_4 oxidation than that of the other two forest soils

at 2nd day of incubation ($P < 0.01$, Fig. 4a). As for the O-horizon, the EBF primary forest significantly increased CH_4 oxidation compared with the two secondary forests of ANF and PBF (Figs. 4b, 4d), and the mean CH_4 oxidation rates by EBF was approximately twice that of ANF and PBF (Fig. 4d). Generally, forest succession stages have significant effects on CH_4 oxidation (Table 2, $P < 0.001$) under the high CH_4 concentration surroundings, and the mean daily rates of CH_4 fluxes were − 0.307 ± 0.033, − 0.305 ± 0.034 and − 0.549 ± 0.067 ng kg^{-1} for early-, mid-, and late-stage respectively (Fig. 4d). On the 1st, 5th, 6th, 7th, and 9th days, the CH_4 oxidation rates were significantly higher in the O-horizon of EBF than that in the secondary forests of ANF and PBF (Fig. 4b).

3.3. Effects of forest succession on the cumulative CH4 fluxes

Forest succession had a significant impact on the cumulative CH_4 fluxes, for both the ambient and potential rates (Figs. 5, 6). The cumulative ambient CH_4 fluxes of subtropical forest was between 0.00295 and − 0.0402 ng kg^{-1} after 3 days incubation (Fig. 5) and the cumulative potential CH_4 oxidation was between − 4.50 and − 2.48 ng kg^{-1} after the 9 days incubation (Fig. 6). As for the A-horizon soil, the cumulative potential CH_4 fluxes in mid stage of PBF was significantly higher than that in the early stage of ANF (−3.57 vs. −2.93 ng kg^{-1} for PBF and ANF respectively, $P < 0.01$, Figs. 6a, 6d), similar pattern was found for the ambient CH4 oxidation as showed in Figs. 5a and 5d. As for the O-horizon, the cumulative potential CH_4 fluxes of the three succession stages was between − 5.88 and − 1.94 ng kg^{-1}, and the CH_4 fluxes in the primary forest EBF was significantly lower than that of the two secondary forests (ANF −2.56, PBF −2.49 and EBF −4.42 ng kg^{-1}, Fig. 6e). When accumulating the amount of potential CH_4 oxidation in the O and the A layer, the CH_4 fluxes in EBF was also significantly lower than that of ANF and PBF (Figs. 6e, 6f).

Depth effects on the cumulative methane fluxes in the same succession stage were performed. The results showed that in the early stage of ANF, there was no significant difference of the cumulative potential CH_4 oxidation between the A- and O- horizons (−2.92 vs. −2.56 ng kg^{-1} for A- and O-horizon respectively, $P > 0.05$, Table 1). However, in the mid stage of PBF, the cumulative potential CH_4 oxidation was significantly
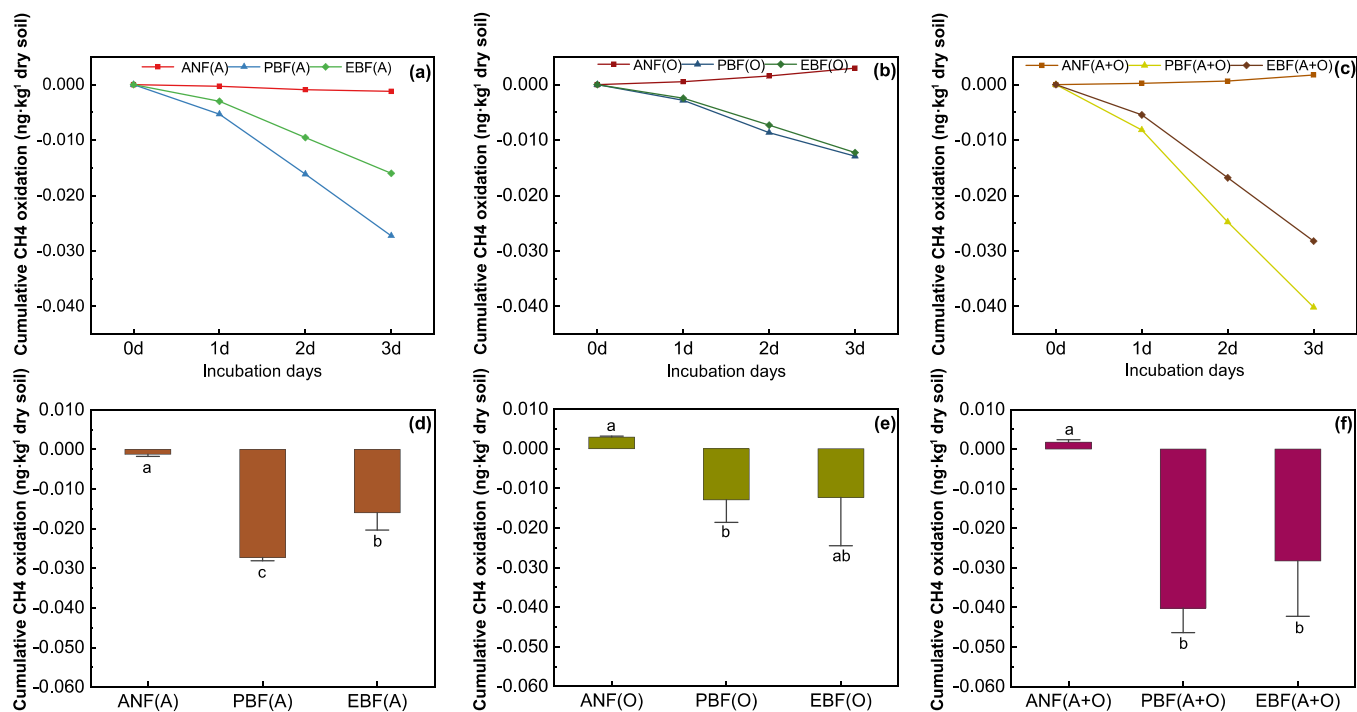
Fig. 5. Effects of forest succession on the cumulative CH₄ oxidation at the original CH₄ concentration of 1.85 ppm. Data are standard error ± mean (n = 8). The dynamic changes of the cumulative ambient CH₄ oxidation in A-horizon (Fig. 5a), O-horizon (Fig. 5b) and the combined horizons of A+O (Fig. 5c), and the average of the cumulative ambient CH₄ oxidation rates in A-horizon (Fig. 5d), O-horizon (Fig. 5e) and the combined horizons of A+O (5 f). Different lowercase letters denote significant differences between the three forests (*P* < 0.05). ANF: Alnus nepalensis forest (early-stage); PBF: Populus bonatii forest (mid-stage), EBF: Evergreen broad-leaved forest (late-stage).
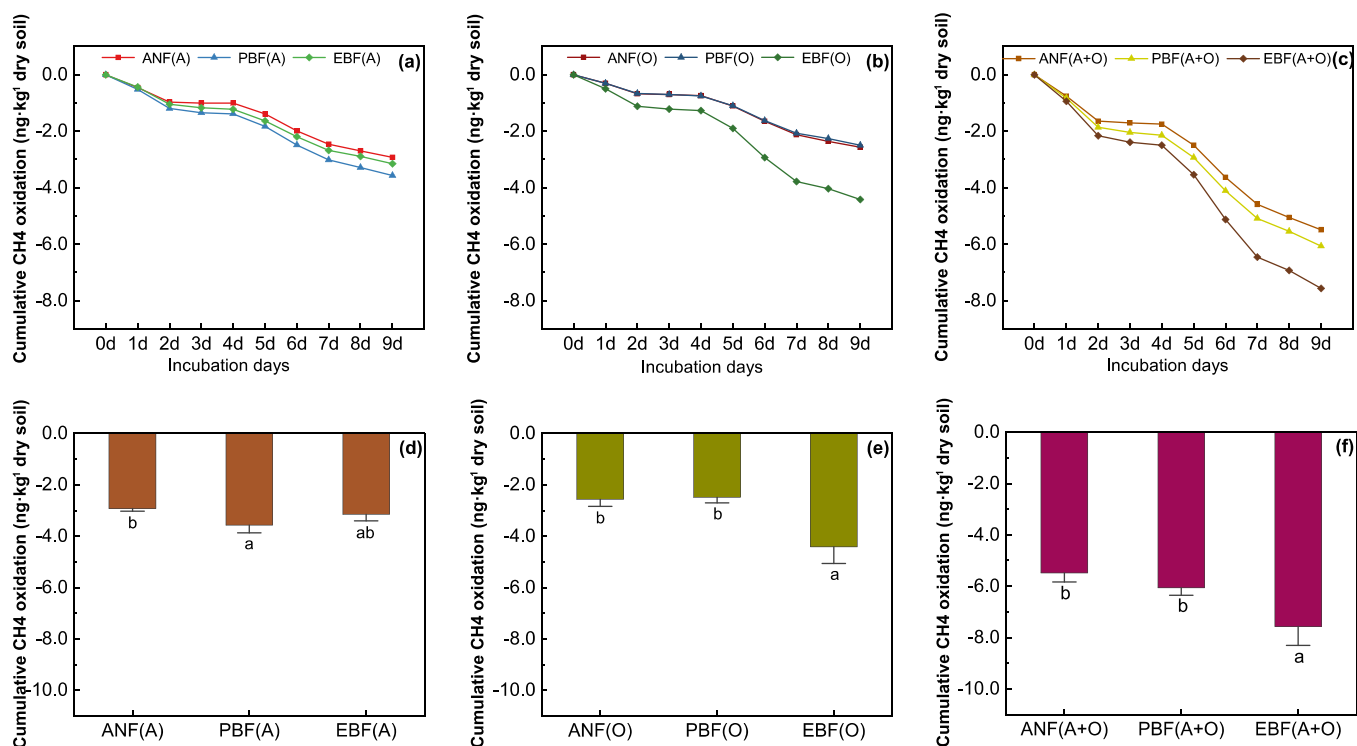

Fig. 6. Effects of forest succession on the cumulative CH₄ oxidation at the original CH₄ concentration of 1000 ppm. Data are standard error ± mean (n = 8). The dynamic changes of the cumulative potential CH₄ oxidation in A-horizon (Fig. 5a), O-horizon (Fig. 5b) and the combined horizons of A+O (Fig. 5c), and the average of the cumulative potential CH₄ oxidation rates in A-horizon (Fig. 5d), O-horizon (Fig. 5e) and the combined horizons of A+O (5 f). Different lowercase letters denote significant differences between the three forests (*P* < 0.05). ANF: *Alnus nepalensis* forest (early-stage); PBF: Populus bonatii forest (mid-stage), EBF: Evergreen broad-leaved forest (late-stage).
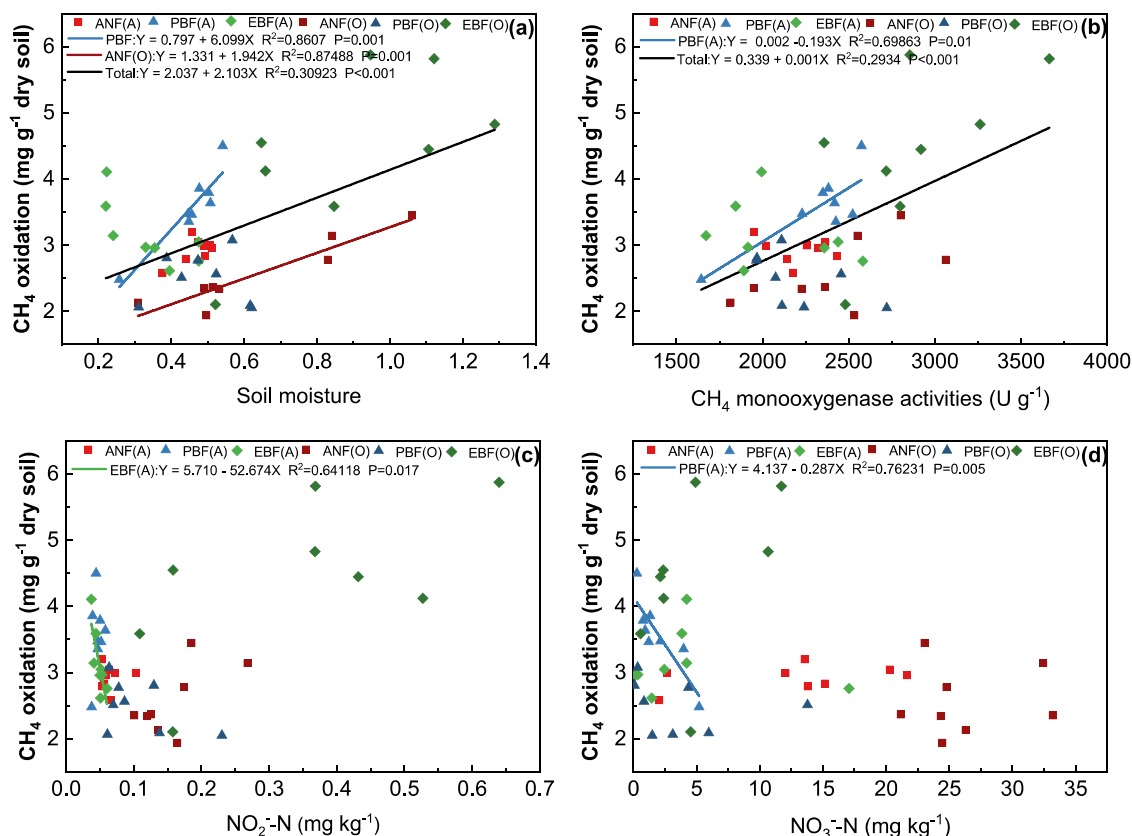
Fig. 7. Relationships between the cumulative CH$_4$ oxidation and (a) soil moisture, (b) soil methane monooxygenase activities, (c) nitrite conent and (d) nitrate content. Regression analyses were either based on the entire dataset from both O- and A-horizons of the three forests of ANF (*Alnus nepalensis* forest, early-stage), PBF (*Populus bonatii* forest, mid-stage) and EBF (Evergreen broad-leaved forest, late-stage), or conducted separately for the three forests and two horizons.

Table 3

Multiple stepwise regression analysis of the dependency of CH4 oxidation in different forest succession stages to potential control factors.

Explained variable		Regression equation	r^2	P	Factors excluded
CH$_4$ oxidation	PBF(A-horizon)	$Y = 1.692 - 31.717NO_2^- + 7.409SM$	0.956	< 0.001	NO$_3^-$, MMO
	EBF(A-horizon)	$Y = 5.71 - 52.674NO_2^-$	0.641	0.017	SM , NO$_3^-$, MMO
	ANF(O-horizon)	$Y = 1.331 + 1.942SM$	0.875	0.001	NO$_3^-$, NO$_2^-$
	ANF(A+O horizons)	$Y = 2.062 + 2.304SM - 5.229NO_2^-$	0.577	0.004	NO$_3^-$, MMO
	PBF(A+O horizons)	$Y = 0.764 - 10.209NO_2^- + 0.001MMO$	0.602	0.003	SM , NO$_3^-$
	EBF(A+O horizons)	$Y = 2.969 - 4.137NO_2^-$	0.562	0.001	NO$_3^-$, MMO , SM
	Total	$Y = 2.037 + 2.103SM$	0.309	< 0.001	NO$_3^-$, NO$_2^-$, MMO

NO$_2^-$: soil nitrite content (mg N kg^{-1} dry soil); NO$_3^-$: soil nitrate content (mg N kg^{-1} dry soil); SM: soil moisture; MMO: soil methane monooxygenase activities (U g^{-1}).

lower in the A-horizon than that in the O-horizon (−3.57 vs. −2.49 ng kg^{-1} for A- and O-horizon respectively, $P < 0.05$, Table 1); while contrast result was found in the late-stage of EBF with the higher cumulative potential CH$_4$ fluxes in the O- layer (−3.15 vs. −4.42 ng kg^{-1} for A- and O- respectively, $P < 0.05$, Table 1).

3.4. Relationships between the potential CH$_4$ oxidation and soil properties

In general, the cumulative potential CH$_4$ oxidation in different succession stages was positively correlated with soil moisture (R^2 = 0.860, $P < 0.01$, Fig. 7a) and MMO activity (R^2 = 0.699, $P < 0.05$, Fig. 7b). Likewise, in some given forest succession stage, we obtained similar results. For example, in the ANF O-horizon and the PBF A-horizon, the amount of CH$_4$ oxidation was positively correlated with soil moisture (Fig. 7a); the amount of CH$_4$ oxidation in the PBF A-horizon was positively correlated with the MMO activity (Fig. 7b). In contrast, the cumulative CH$_4$ oxidation was negatively correlated with soil nitrite and nitrate content but only within the specific successional stages (Figs. 7c, 7d).

Stepwise regression analysis was used to identify the most important variables controlling CH$_4$ oxidation at different succession stages. In the early stage of succession (ANF), CH$_4$ oxidation was mainly controlled by soil moisture and nitrite content, which together explained 57.7% of the CH$_4$ oxidation (O- and A- horizons), while if only the O- horizon was considered, moisture explained 87.5% of the CH$_4$ oxidation (Table 3). In the mid stage of PBF, the results were similar, i.e., CH$_4$ oxidation at A horizon was mainly affected by soil moisture and nitrite content, which together explained 95.6% of CH$_4$ oxidation, while if both the two horizons were conducted the CH$_4$ oxidation was mainly affected by the MMO activity and nitrite content (Table 3). At the late stage of EBF, CH$_4$ oxidation was mainly affected by soil nitrite content, and the explained ability was 56.2–64.1%. Overall, soil moisture was the main factor controlling the subtropical forest CH$_4$ oxidation (R^2 = 0.309, $P < 0.001$, Table 3).

4. Discussion

In this study, experiments on ambient and potential methane
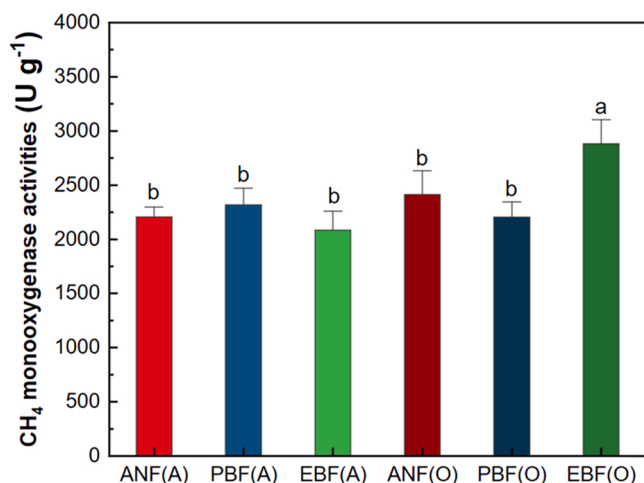
Fig. 8. Soil methane monooxygenase activities in O- and A-horizons of the three forest succession stages. Note: Different lowercase letters denote significant differences between the three forests and two layers ($P < 0.05$). ANF: *Alnus nepalensis* forest (early-stage); PBF: Populus bonatii forest (mid-stage), EBF: Evergreen broad-leaved forest (late-stage).

oxidation were conducted by collecting O- and A-horizons from the three successional stages of subtropical forest. We found that O- and A-horizon soils in different succession stages varied in the methane oxidation capacity. For the A- and O-horizons in general, the potential methane oxidation capacity increased with the forest maturity/succession, especially in the O-horizons, where the trend was more pronounced (Fig. 6 f). Our results are consistent with previous reports, for example, the study by Zhou (et al. (2019) illustrated that soil methane oxidation accelerated with the stand age in a secondary subtropical forest of southwest China. In soils of eastern Amazonia tropical forest, the annual CH_4 oxidation in the primary forest was two times higher than that in the secondary forest (2.1 vs 1.0 kg CH_4 ha^{-1} y^{-1} for primary and secondary forest respectively) (Verchot et al., 2000). However, there is a study showed that CH_4 oxidation in the oak forest enhanced with stand age but declined with age for soils under Norway spruce (Christiansen and Gundersen, 2011). In the UK Gisburn forest, observations from the shallow organic soils showed that stand age had no significant effect on soil CH_4 oxidation (McNamara et al., 2008), indicating that the effects of stand age on CH_4 oxidation is not always consistent and still is uncertain (Tate, 2015). In the present study, the

variations of soil CH_4 oxidation in the three forest succession stages were found to be related with the soil moisture. Many studies showed that forest soil CH_4 oxidation was negatively correlated with soil moisture, because high moisture states limit the diffusion of atmospheric CH_4 at the soil-atmosphere interface, which led to a decrease in the substrate concentration of methanotrophs in the soil, and accordingly the methane oxidation rates decreased (Bhardwaj and Dubey, 2020; Feng et al., 2020). However, our results were opposite and may be due to the moisture status at the time of sampling. During the dry seasons in subtropical forest as we sampled, soil microbial activity was limited by the relatively low moisture condition (the gravimetric moisture: 30 ~ 65% at most plots), while the high canopy density of the primary forest was not conducive to the evaporation of soil water compared with the secondary forests, and thus the relatively wet conditions in the primary forest during the dry seasons was beneficial to the soil microbial activity, including the activity of methanotrophs (Knief, 2019). This inference was supported by the results of soil respiration as shown in Fig S1, with the similar pattern of cumulative CO_2 fluxes compared to the patterns of the cumulative CH_4 oxidation (Fig S1). It should be noted that our sampling this time was the dry season and if in the rainy season, when the soil moisture was largely increased, the more anaerobic microsites might be created, which was conducive to the production of methane by methanogenesis. Another explanation might be due to the differences between the intact and cultured soils. We did not use the intact soil although we did not sieve the soil after taking back to the laboratory to avoid disturbance, the overall structure of the soil may still be affected, especially the change of porosity, which affects the use of water and the oxidation of methane by soil microorganisms.

In the organic layer, we found that the late-stage of EBF had the strongest potential CH_4 oxidative ability, which was significantly higher than that of the early- and mid-successional stages (Figs. 4b and 4d, Fig. 6). It has been reported that the presence of humus in organic layer reduces the rate of soil methane oxidation (Dong et al., 1998; Saari et al., 1998), but we observed significant methane oxidation in the organic (humus) layers at all three succession stages, indicating that the subtropical forest O-horizon also acts as a methane sink. The study by Dong et al. (1998) and Saari et al. (1998) was conducted in coniferous forests, where the organic compounds of monoterpenes in humic substances can inhibit the oxidation of CH_4, while the inhibitory effect of this organic matter may not exist in broad-leaved forests (Tate, 2015). The methane oxidation of O-horizon in EBF was stronger than that of the other two forests O-horizon (Fig. 4d), which could be due to the higher enzyme activities of methane monooxygenase in the EBF O-horizon (Fig. 8), promoting the CH_4 oxidation.
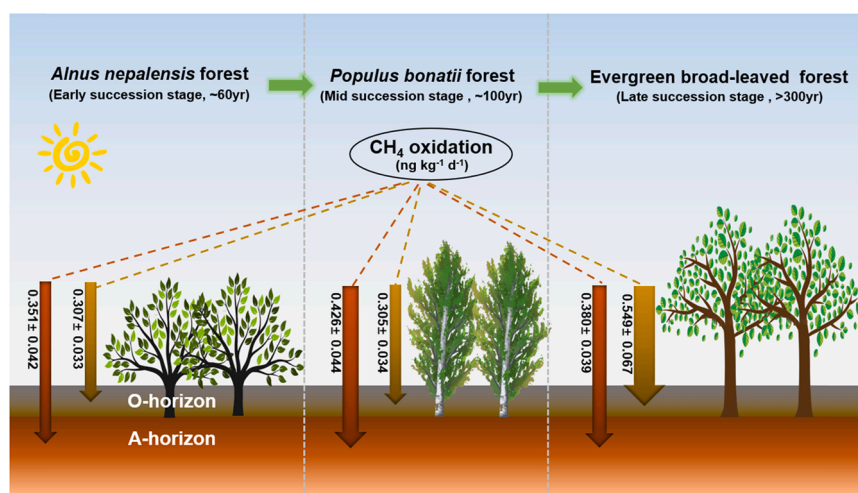

Fig. 9. Scheme of the CH_4 oxidation capacity in O- and A-horizons under the successional stages of subtropical forests. Thickness of the arrows is representative for the respective CH_4 oxidation rates.

In addition to the controlling factor of soil moisture, the higher CH_4 oxidation rate occurring in the late-successional stage of EBF might be attributed to the declines of soil inorganic N (NH_4^+, NO_2^- and NO_3^-) availabilities in comparison to the early-stage of ANF and mid-stage of PBF. In the early stage of forest succession, the decline of plant communities and the simplification of species will reduce the oxidation of inorganic N by plants, resulting in the increase of soil inorganic nitrogen (Templer et al., 2008). With the development of vegetation succession and the restoration of plant diversity, more soil inorganic nitrogen is retained by the plant and thus causing its decrease (Zhang et al., 2013). Our results of regression analysis revealed that soil NO_2^- and NO_3^- rather than NH_4^+ was negatively correlated to the CH_4 oxidation across the forest successions (Table 3, Fig. 7), indicating that NO_2^- and NO_3^- production by nitrification was strong, especially at the early stage of ANF forest and might inhibit the CH_4 oxidation. In addition, the transformed NO_3^- and NO_2^- produced via NO_3^- reduction are probably toxic to CH_4-oxidizing bacteria (Conrad, 2009). Similarly, previous studies reported that high NO_3^- concentrations has been found as a strong inhibition of CH_4 oxidation in unsaturated forest soils, and this inhibition effects varied with the vegetation types (Fender et al., 2012; Jang et al., 2011; Mochizuki et al., 2012). Our results, together with those from other reports, suggest that the decrease in soil NO_2^- and NO_3^- contents may be as one of the key mechanisms that regulate the increases in soil CH_4 oxidation along the successional gradients of subtropical forests. (Fig. 9).

Beside the above mentioned factors, the increased plant diversity with the forest succession and associated changes in monterpene release can also affect changes in soil CH_4 oxidation (Dubois et al., 2018; Song et al., 2022; Tate, 2015; Zhu et al., 2021). In the process of forest succession, the role of soil nutrient limitation is strengthened with the development of aboveground vegetation (Li et al 2013; Xu et al., 2022; Yang et al., 2022), which promotes the distribution of plant photosynthesis products to roots to facilitate the absorption of nutrients, resulting in an increase in plant root biomass and exudates (Zhang et al., 2013; Prescott et al., 2020; Maarastawi et al., 2018), thereby increasing the soil microbial biomass and enzymatic activities, including the number of methanotrophs (Wu et al., 2021; Wang et al., 2022) and the shift between oligotrophic and copiotrophic methanotrophic communities (Dorr et al., 2010;). In the same study area, the previous studies showed that the existing amount and carbon and nitrogen components of litter in the three forest succession stages changed significantly, which affected the community characteristics of soil arthropods and nematodes (Zhao et al., 2011, Li et al., 2016). Together with our study, we inferred that the vegetation and litter composition shifts along the forest succession might change nutrient status and thus drive the methanotrophs communities and patterns of CH_4 oxidation. The powerful molecular techniques in the past decade have provided new insights into soil CH_4 emission mechanisms and the characteristics and population functions of Type I and Type II methanotrophs (Tate, 2015; Knief et al., 2015;). In this study, we also attempts to use the quantitative PCR and high-throughput sequencing technologies to reveal the abundance, diversity and gene expression of methanotrophs, but unfortunately we failed to amplify Type I and Type II methanotrophs based on published primers (Cai et al., 2016b). It might be related to the strong inhibition of DNA polymerase in the PCR by high humic acid in forest humus and soil although we have tried several methods for DNA extraction and purification. The researches on soil methanotrophs and methanogenesis were mostly focused on paddy fields and wetlands (Jiang et al., 2019), and the studies on the CH_4-related gene expression in forests was rare, but this is critical to reveal the CH_4 emission mechanisms, and thus it need to be considered in the future.

5. Conclusions

Our study showed the exclusively CH_4 sink across the early-, mid-, and late stages of forest succession and the significant vertical distributions of CH_4 oxidation. The highest oxidation rate was observed in the organic horizon of the late-successional stages, which increased the CH_4 oxidation by 29% and 21% respectively compared with the early- and mid-successional stages. Furthermore, our study revealed that soil moisture and methane monooxygenase activity were the facilitator of soil CH_4 oxidation while soil nitrite and nitrate availability probably inhibit the CH_4 oxidation along forest successional gradients. Since primary and secondary forests are the main types of forest cover in the global and subtropical regions, our study indicating that the vegetation shifts along the forest succession might change the litterfall composition and nutrient status and consequently drive the patterns of CH_4 oxidation. Understanding the CH_4 oxidation dynamics and the controlling factors in the subtropical forest succession is essential to improve our knowledge of their role in the global carbon cycle and to mitigate and adapt to climate change.

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.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psep.2022.12.064.

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