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Urbanization degree rather than methanotrophic abundance decreases soil CH_4 uptake

Mingyue Zhang [a], Shimei Weng [a], Hailun Gao [a], Lan Liu [a], Junxiang Li [b], Xiaoqi Zhou [a,*]

[a] Zhejiang Tiantong Forest Ecosystem National Observation and Research Station, Center for Global Change and Ecological Forecasting, School of Ecological and Environmental Sciences, East China Normal University, Shanghai 200241, China
[b] School of Design, Shanghai Jiao Tong University, Shanghai 200240, China

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ABSTRACT

Urbanization has been increasing worldwide, which can greatly influence ecosystem functions such as soil methane (CH_4) uptake. However, little information is available for quantifying the effects of urbanization on soil CH_4 uptake and the underlying microbial mechanisms. By conducting a *meta*-analysis of soil CH_4 uptake under urbanization worldwide in combination with measurements of potential soil CH_4 uptake from 134 samples along an urbanization gradient across Shanghai, China, we found that urbanization significantly decreased soil CH_4 uptake with greater inhibition under higher urbanization levels. Urbanization degree, index of urbanization levels, explained large variations of soil CH_4 uptake, and urbanization decoupled the relationships between methanotrophic abundance and soil CH_4 uptake, which was often neglected in previous research. To our knowledge, this is the first study to quantify the effects of urbanization degree on soil CH_4 uptake, which need to be incorporated into Earth system models for estimating the global CH_4 budget more accurately.

1. Introduction

Urbanization has been increasing worldwide, with considerable growth in developing countries (Berry et al., 2008). In 1900, a mere 10% of the global population were urban dwellers, but now nearly 4% of the terrestrial area has been urbanized and >50% of the global population lives in urban areas (Ciesin, 2004). Increasing population densities and urban sprawl have caused land uses to change from natural forest ecosystems into smaller, urban residential properties with impervious road surfaces (IPCC, 2013). Studies have shown that urbanization can greatly influence ecosystem functions (Groffman and Pouyat, 2009; Chen et al., 2014).

As it is an important ecosystem function, soil methane (CH_4) uptake in urban ecosystems has received much attention, given that CH_4 is the second most important greenhouse gas after CO_2, contributing about 25% to global warming (IPCC, 2013). Terrestrial ecosystems represent the largest biological sink for atmospheric CH_4, but urbanization can impair soil CH_4 uptake, which, in turn, results in positive feedback to global warming (IPCC, 2013). Previous studies have reported that compared with rural areas, urbanization significantly decreased soil CH_4 uptake by ~ 44% (Zhang et al., 2015) in Southern China or by ~ 86% (Groffman and Pouyat, 2009) in the eastern USA.

Soil CH_4 uptake is mediated by specific microorganisms called methanotrophs, which are obligately aerobic, Gram-negative bacteria (Murrell, 2010). Based on differences in their physiological, biochemical, and morphological properties, methanotrophs can be divided into low-affinity methanotrophs and high-affinity methanotrophs, the latter being responsible for atmospheric CH_4 consumption (Hanson and Hanson, 1996; Tate, 2015). The key step in soil CH_4 oxidation is catalyzed by CH_4 monooxygenase (MMO), which converts CH_4 into methanol in the CH_4 oxidation pathway. The monooxygenase enzyme has two forms, i.e., particulate CH_4 monooxygenase (pMMO) and soluble CH_4 monooxygenase (sMMO) (Hanson and Hanson, 1996; Kolb, 2009). As the majority of methanotrophs harbor pMMO, the *pmoA* gene that encodes the β-subunit of pMMO has been widely used to target soil methanotrophic communities in terrestrial ecosystems (Kolb, 2009; Murrell, 2010). Previous studies have reported that soil CH_4 oxidation rates can be greatly influenced by biotic factors such as methanotrophic communities and abiotic factors such as soil temperature and moisture contents (Tate, 2015). Some studies have demonstrated that the methanotrophic community structure and abundance have a notable effect on soil CH_4 efflux (Martins et al., 2017; Lafuente et al., 2019). However, researchers have also demonstrated that soil properties can, to some extent, exert more influence on soil CH_4 uptake than the methanotrophic

* Corresponding author.
E-mail address: xqzhou@des.ecnu.edu.cn (X. Zhou).

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community structure and abundance in terrestrial ecosystems (Kou et al., 2017). Importantly, the relative contributions of these factors to soil CH_4 uptake are strongly scale-dependent across grassland ecosystems (Kou et al., 2017).

Urbanization can strongly influence soil CH_4 uptake through two different pathways. On one hand, urbanization can directly influence soil CH_4 uptake through the soil temperature as a result of the urban heat island effect (Oke and Maxwell, 1975; Gregg et al., 2003). On the other hand, urbanization can indirectly influence soil CH_4 uptake by altering soil properties as well as the community structure and abundance of methanotrophs during the process of land use changes (Li et al., 2013). For example, in urban regions, natural forest ecosystems are replaced by residential areas, traffic infrastructure, intensively managed green spaces, and public lands like schools and hospitals (IPCC, 2013; Li et al., 2013). Until now, many studies have only compared soil CH_4 uptake in urban and suburban regions. However, few studies have quantified the effects of urbanization levels on soil CH_4 uptake and the underlying microbial mechanisms.

We first carried out a *meta*-analysis to quantify the effects of urbanization level on soil CH_4 uptake worldwide. On the basis of this literature review, we found that there were limited studies focusing on soil CH_4 uptake under urbanization. To our surprise, we did not find any mention of the associated methanotrophic communities related to soil CH_4 uptake in urban contexts. To fill this knowledge gap, we selected 134 soil samples along a gradient of urbanization across Shanghai, one of the largest metropolitan cities worldwide (Li et al., 2013) and quantified the effects of urbanization on soil CH_4 uptake. Overall, the objectives of the study were to investigate (1) the influence of the degree of urbanization, soil properties and methanotrophic communities on soil CH_4 uptake; and (2) the key drivers influencing soil CH_4 uptake under urbanization worldwide.

2. Materials and methods

2.1. Meta-analysis of the effects of urbanization on soil CH_4 uptake worldwide

We systematically searched all peer-reviewed journal articles using Web of Science with the following search term combinations: (urbanization or urban to rural gradient) AND (methane OR CH_4 uptake). We reviewed all the articles revealed by the search and selected those that met the following criteria: (1) the study was conducted with in situ field measurements or laboratory incubation; and (2) soil CH_4 uptake could be extracted directly from the texts, tables, and figures. When one publication included several sites under different urbanization levels, such as suburban or urban areas, we considered them to be different observations. In total, we found 103 observations at seven sites from five papers (Table S1). For each study, we collected the latitude, longitude, mean annual temperature and mean annual precipitation. We used GetData to extract data digitally from the figures when the results were reported graphically. Control and treatment means, standard deviations and sample sizes (n) of CH_4 were extracted directly 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 by using the methods described by Zhou et al. (2017) and Feng et al. (2020). Briefly, ln$R$ was calculated as follows:

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

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 lnRR was calculated as:

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

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. Study area and experimental design in Shanghai

We selected Shanghai City, one of the largest metropolitan cities in China, to investigate the effects of urbanization on soil CH_4 uptake and its driving factors. Shanghai is located in Eastern China (30°40′–31°53′N, 120°52′–122°12′E) and the region is dominated by a typical subtropical monsoon climate, with a mean annual temperature of 16.0 °C and a mean annual precipitation of 1158.1 mm (Li et al., 2013). The area of Shanghai is 6340.5 km^2, with a longitudinal extent of 120 km and a latitudinal extent of 100 km. As the commercial and financial center of China, according to the Shanghai municipal statistics bureau, Shanghai had a total gross domestic product of 2.16 trillion RMB (US$ 352.36 billion) and a total of population of 23.80 million in 2012 (Liu et al., 2019). Shanghai has experienced rapid economic development since the beginning of the 1990s, representing the highest degree of urbanization in China (Liu et al., 2019). In this region, the representative vegetation type is subtropical evergreen broadleaf forest.

We divided the whole Shanghai area into ~ 700 cells with a grid network of 3 km × 3 km (Fig. 2). Of these, we selected two 18-km-wide transects across the city center, one oriented east–west and the other oriented south–north. With the exception of impervious surfaces and water (such as lakes and rivers), we randomly established 134 sites based on Google Earth (Fig. 2). For each site, the land use type was classified and the degree of urbanization was calculated from the percentage of each urban land use type within the total area of the circle (1 km radius) around each sample plot (Wang et al., 2020). We classified the 134 sampling sites into three urbanization levels based on urbanization degree: low urbanization level (urbanization degree < 0.3), moderate urbanization level (0.6 > urbanization degree > 0.3) and high urbanization level (urbanization degree > 0.6) (Mckinney, 2002). In this study, land use types were classified into the following five categories: green land (Gre), including forests, shrubs, grass lands, parks, and greenbelts; agricultural land (Agr), including farmland, vegetable plots and orchard; residential land (Res), including old houses, buildings, garden houses, villas and rural residence; transport land (Tra), including different grades of roads, railways, and public transportation depots; and public land (Pub), including public facilities like administrative organizations, hospitals, schools, and research institutions. Moreover, as there was only one industrial site, we classified it into the Pub group and analyzed this group together across land uses.

In addition, we also selected an adjacent typical subtropical evergreen broadleaf forest as an ambient control at Tiantong National Forest Park, Ningbo City, Zhejiang Province (29°52′N, 121°39′E) (Bu et al., 2018). 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).

2.3. Soil sampling and measurements of soil physicochemical properties in Shanghai

At each sampling site, we randomly selected a 50 m × 20 m plot, then three 1 m × 1 m subplots were established within each plot, with at least 5 m spacing between adjacent subplots. Within each subplot, we used a soil auger (2.5 cm in diameter) at a depth of 0–10 cm following a diagonal sampling pattern (i.e., one point at each corner and one in the center of each plot). The soil cores within each subplot were immediately mixed thoroughly and kept in a cooler at 4 °C. In addition, we also collected soil samples from the adjacent subtropical forest based on the same procedure. All soil 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.

Soil moisture content was determined gravimetrically after the samples were oven-dried at 105 °C overnight. Soil pH was measured at a 1:2.5 dry soil-to-water ratio. Soil TC and TN contents were determined on a Vario MICRO cube elemental analyzer (Elementar, Germany) (Bu et al., 2018). The NH_4^+–N and NO_3^-–N contents were measured with a Smartchem Discrete Auto Analyzer (Smartchem 200, AMS, Italy) (Gu et al., 2019). We also calculated the ratio of TC to TN (C to N ratio) to represent the quality of the soil (Bu et al., 2018).

2.4. Measurements of potential soil CH_4 uptake under urbanization

All the soil samples were incubated in the laboratory to measure their potential soil CH_4 uptake with ambient air containing ~ 2 parts per million (ppm) CH_4 (Kou et al., 2017; Gu et al., 2019). About 15 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 21 days. During the incubation, we collected gas samples once a week. Headspace gas samples (approximately 30 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). Potential soil CH_4 uptake in each flask was calculated from the differences in the headspace CH_4 concentration over the incubation time (Gu et al., 2019). Finally soil CH_4 uptake was expressed as g CH_4 ha^{-1} day^{-1} based on the soil bulk density data of each sample site.

2.5. Soil genomic DNA extraction and real-time PCR of the pmoA gene

Soil genomic DNA was extracted from 0.5 g of each sample by following procedures described previously (Zhou et al., 2010). The abundance of the *pmoA* gene of methanotrophs was quantified via real-time quantitative polymerase chain reaction (PCR) with the primers A189F (5′–GGNGACTGGGACTTCTGG–3′) and A650R (5′–ACGTCCTTACCGAAGGT–3′), which can target high-affinity methanotrophs (Gu et al., 2019; Lafuente et al., 2019). All real-time quantitative PCR reactions were performed with the ViiA 7 Real-Time PCR System (Applied Biosystems, CA, USA) (Wcgene Biotechnology, Shanghai, China). The qualitative PCR mixture (10 μL) was as follows: 5 μL 2 × TB Green Premix Ex TaqII(Tli RNaseH Plus), 0.2 μL 50 × ROX Reference Dye, 0.4 μL of each primer, 1 μL of DNA template and 3 μL ddH_2O. We constructed plasmids containing the cloned *pmoA* gene fragments, and used multiple successive 10-fold serial dilutions of the plasmids as DNA templates, then amplified them by real-time fluorescent quantitative PCR. The real-time PCR program had the following settings: initial denaturation at 95 °C for 30 s, followed by 50 cycles of denaturation at 95 °C for 15 s, annealing at 55 °C for 30 s and elongation at 72 °C for 30 s, and then a final elongation step at 72 °C for 5 min. The melting process was automatically generated by the ViiA 7 Real-Time PCR System. We took the logarithm of the DNA template concentrations as the x-axis, and the detected threshold cycles, i.e., CT values, as the y-axis to obtain a standard curve based on a linear regression equation. Then we put the CT values of the test samples into this equation to calculate the *pmoA* gene concentrations in the samples. The amplification efficiency of quantitative PCR can be estimated from the slope of the standard curve. During the process, the amplification efficiency of PCR was 83.2% with an R^2 value of 99.5% for the standard curve.

2.6. Statistical analysis

For the *meta*-analysis of the effects of urbanization on soil CH_4 uptake worldwide, a categorical random effect model was used to assess whether CH_4 uptake showed different responses to different urbanization levels. All statistical analyses were performed in MetaWin 2.1.

For the study quantifying the effect of urbanization on soil CH_4 uptake in Shanghai, we first used one-way analysis of variance (ANOVA) to determine the effect of land use types on soil physicochemical properties, potential soil CH_4 uptake and *pmoA* gene copies. Secondly, we compared the differences in potential soil CH_4 uptake and *pmoA* gene copies among different urbanization levels. Pearson's correlation analysis was performed to test the relationships of potential soil CH_4 uptake with urbanization degree, methanotrophic abundance (*pmoA* gene copy numbers) and soil physicochemical properties. For the soil methanotrophic abundance data from each soil sample, logarithmic transformation was performed for analysis. All results were tested statistically via ANOVA and Tukey's test, and differences were considered significant at $P < 0.05$ between treatments. All ANOVA and correlation analyses were performed in R (R Core Team, 2014).

Structural equation modeling was performed with R software with the *lavaan R* package to explore the causal links among potential soil CH_4 uptake, urbanization degree, methanotrophic abundance and soil properties under different land uses and under different urbanization levels. In structural equation modeling, a χ^2 test is used to determine whether the covariance structures implied by the model adequately fit the actual covariance structures of the data. A non-significant χ^2 test ($P > 0.05$) indicates an adequate model fit. The coefficients of each path, taken as the calculated standardized coefficients, were determined by analyzing the correlation matrices. Paths in this model were considered to be significant at $P < 0.05$. These coefficients indicate by how many standard deviations the effect variable would change if the causal variable was changed by 1 standard deviation (Zhou et al., 2017). In addition, we calculated the standardized total effects on soil CH_4 uptake by the sum of the direct and indirect effects of individual key factors on the basis of the structural equation models (Attard et al., 2011).

3. Results

3.1. Effects of urbanization on soil CH_4 uptake worldwide

According to the literature review, urbanization significantly decreased soil CH_4 uptake by 35.4%, with the 95% confidence interval (CI) being between −0.52 and −0.36 (Fig. 1). However, these studies

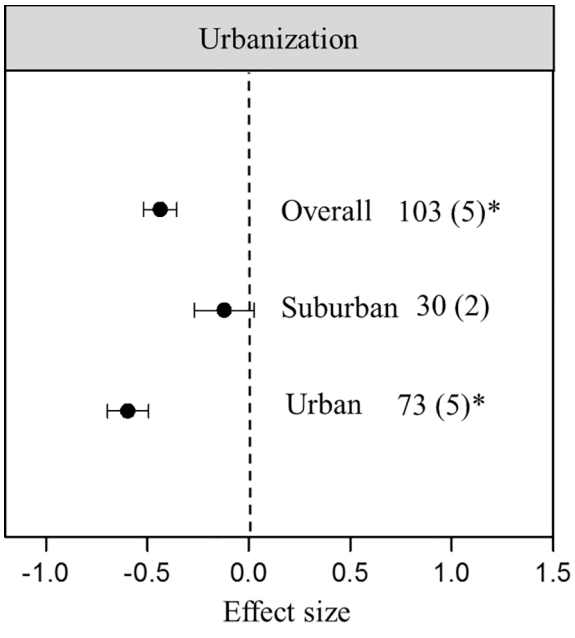

Fig. 1. The mean effect sizes of urbanization on soil CH_4 uptake worldwide. The data are categorized into urban areas and suburban regions. Error bars represent 95% confidence intervals. The dashed line was drawn at a mean effect size = 0. The effect was considered to be significant if the 95% CI of the effect size did not overlap zero. *$P < 0.05$. Number values for each bar indicate the sample size and numbers in brackets indicate the number of experiments.

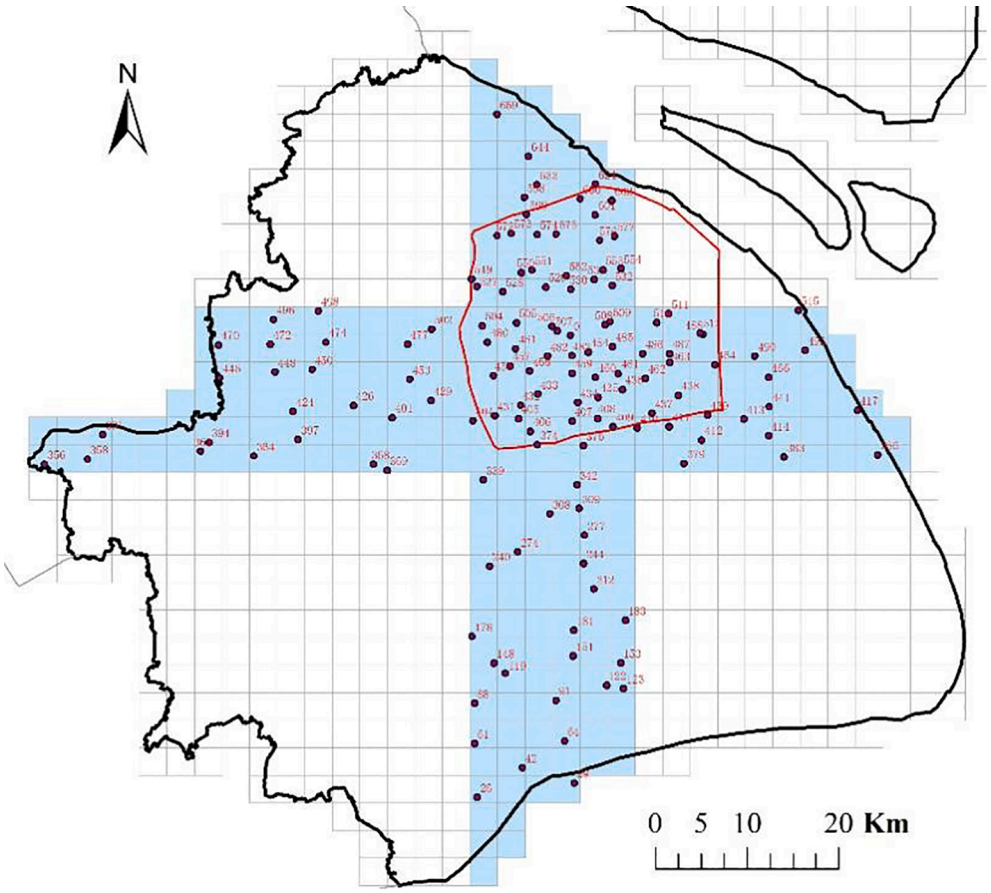

Fig. 2. The 134 sampling sites with a 3 km × 3 km grid along a gradient of urbanization in Shanghai, China. The black line depicts the city boundary and the red line depicts the boundary of the city center. The blue area indicates the two transects, one oriented east–west and the other oriented north–south, both of which cut across the city center. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

only investigated soil CH_4 uptake in urban and suburban regions (Table S1). We found that compared with nearby rural regions, there were no differences in soil CH_4 uptake in suburban regions, whereas urban regions significantly decreased soil CH_4 uptake by 44.9% (95% CI: −0.70 to −0.49).

3.2. Changes in potential soil CH_4 uptake under urbanization in Shanghai

In the literature review, to our surprise, there were no studies that quantified the effects of urbanization on soil CH_4 uptake. To fill this knowledge gap, we collected soil samples from Shanghai City to quantify the effects of urbanization on soil CH_4 uptake. Compared with the ambient control of the adjacent subtropical forest, urbanization significantly decreased potential soil CH_4 uptake (Fig. 3a). In general, urbanization decreased potential soil CH_4 uptake by 37%, 41%, 43%, 29% and 51% in green land (Gre), agricultural land (Agr), residential land (Res), transport land (Tra) and public land (Pub), respectively. Under urbanization, Tra had the highest potential soil CH_4 uptake, followed by Gre, Agr and Res, whereas Pub had the lowest potential soil CH_4 uptake (Fig. 3a). Furthermore, we found that a high urbanization level significantly decreased potential soil CH_4 uptake compared with low and moderate urbanization levels (Fig. 3b). Under low urbanization, we found that Tra had the highest potential soil CH_4 uptake, which was significantly higher than that under Agr, whereas Gre and Pub had similar potential soil CH_4 uptake (Fig. 3c). Under moderate urbanization, Tra, Res and Gre had significantly higher potential soil CH_4 uptake than Agr and Pub (Fig. 3d). Under high urbanization, Tra, Res and Gre had similar potential soil CH_4 uptake, which was significantly higher than that under Pub (Fig. 3e). We need to note that there were no soil samples in Res under low urbanization or in Agr under high urbanization.

3.3. Changes in soil properties and methanotrophic abundance under urbanization in Shanghai

Compared with the ambient control, urbanization significantly decreased soil total C (TC), total N and NH_4^+–N contents ($P < 0.05$, Table 1), but it significantly increased soil pH and NO_3^-–N content under urbanization ($P < 0.05$, Table 1). In general, we found that Gre and Agr had higher soil TC, TN and NO_3^-–N contents, whereas Res and Pub had lower soil TC and TN contents across soil samples along a gradient of urbanization (Table 1). Agr had the lowest NH_4^+–N contents, but the other land uses had similar NH_4^+–N contents (Table 1). We divided all soil samples into three urbanization levels and found that the changes in soil TC, TN and NO_3^-–N contents exhibited a decreasing trend, whereas soil pH and C:N ratios showed an increasing trend along a gradient of urbanization (Table 1). Soil samples under low urbanization had significantly higher TC, TN and NO_3^-–N contents than those under moderate- and high urbanization levels. In contrast, pH and C:N ratios were significantly lower under low urbanization than under the other two urbanization levels. NH_4^+–N contents were similar across all urbanization levels (Table 1).

Unlike soil properties, compared with the ambient forests, urbanization slightly increased soil methanotrophic abundance across land uses in Shanghai (Fig. 3a). Under urbanization, Res showed the highest methanotrophic abundances (mean value of 15.0×10^4 *pmoA* gene copies g^{-1}), which was significantly higher than that under Gre, although methanotrophic abundance was similar among Pub, Agr and

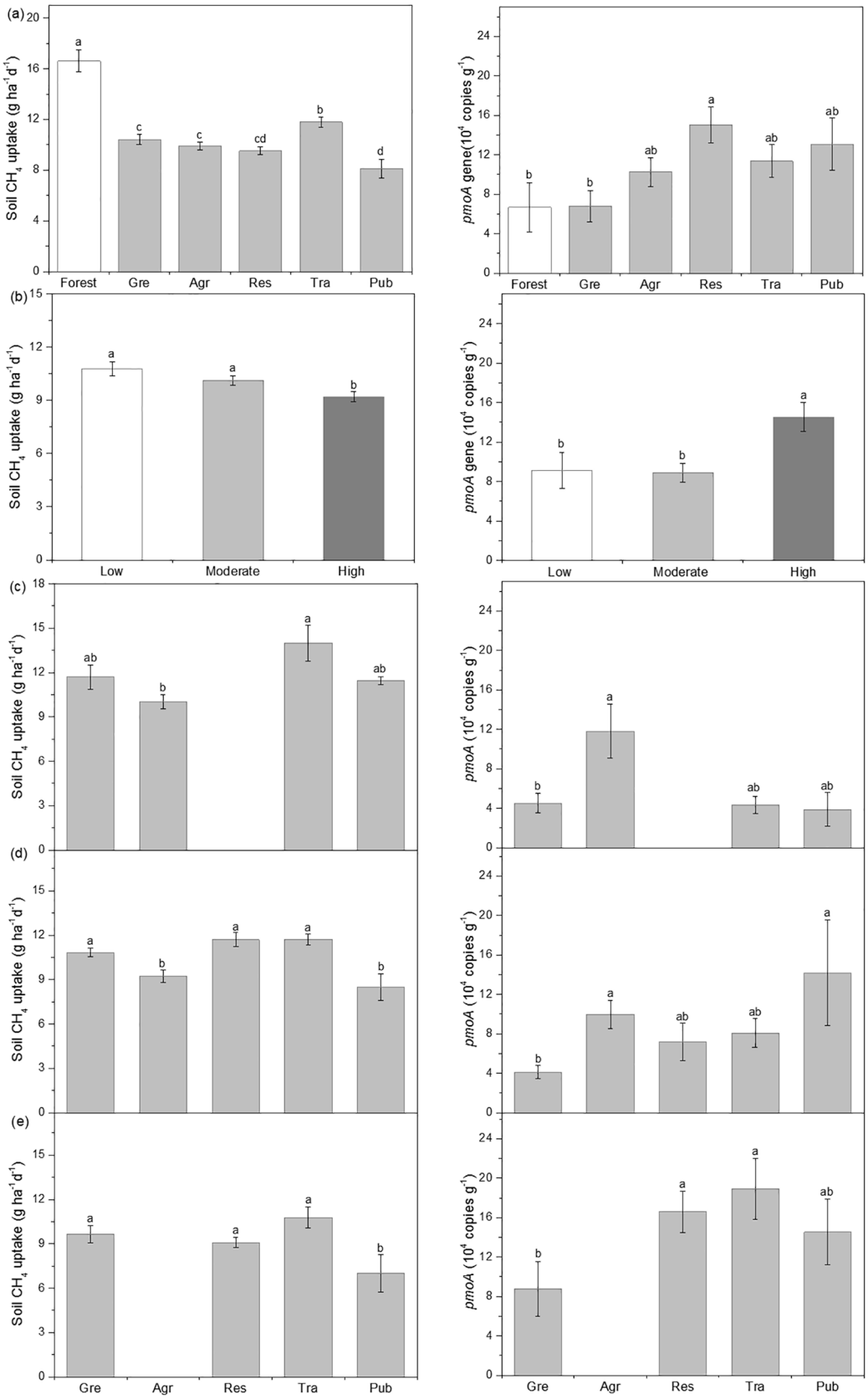

Fig. 3. Changes in potential soil CH_4 uptake (left) and methanotrophic abundance (right) from sampling sites under different land uses (a) and under different urbanization levels (b), as well as at low (c), moderate (d), and high (e) urbanization in Shanghai. In addition, we selected an adjacent forest to act as a representative plant community in this region (a). Values are means ± standard errors. Different letters in the same plot indicate significant differences at $P < 0.05$ among the treatments. The missing bars for the low and high urbanization levels are because of the lack of soil samples in Res at a low urbanization level or in Agr for a high urbanization level. Gre, green land; Agr, agricultural land; Res, residential land; Tra, transport land; Pub, public land. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Soil physicochemical properties in a subtropical evergreen forest and under different urbanization levels as well as different land uses in Shanghai.

Soil properties	Forest	All	Low	Moderate	High	Gre	Agr	Res	Tra	Pub
Moisture (%)	46.65 ± 1.46	22.42 ± 0.31	21.38 ± 0.82b	21.65 ± 0.57b	23.47 ± 0.36a	23.86 ± 0.70a	21.20 ± 0.65b	23.12 ± 0.44a	22.27 ± 0.55ab	21.26 ± 1.30bc
pH	4.50 ± 0.02	7.83 ± 0.03	7.35 ± 0.07b	7.95 ± 0.03a	8.00 ± 0.02a	7.73 ± 0.08b	7.60 ± 0.05c	8.04 ± 0.02a	8.04 ± 0.03a	7.99 ± 0.11a
TC (g kg^{-1})	43.66 ± 3.27	22.36 ± 0.45	24.76 ± 0.94a	21.34 ± 0.82b	21.78 ± 0.65b	25.98 ± 0.93a	23.07 ± 0.95b	20.39 ± 0.63c	20.90 ± 0.83bc	18.74 ± 1.81c
TN (g kg^{-1})	1.91 ± 0.10	1.15 ± 0.02	1.39 ± 0.05a	1.15 ± 0.05b	1.03 ± 0.03c	1.26 ± 0.04ab	1.29 ± 0.05a	0.99 ± 0.02 cd	1.12 ± 0.04bc	0.88 ± 0.07d
C:N	22.76 ± 0.52	19.51 ± 0.16	17.80 ± 0.18c	18.61 ± 0.21b	21.01 ± 0.25a	20.64 ± 0.31ab	17.87 ± 0.16c	20.40 ± 0.28b	18.72 ± 0.37c	21.13 ± 0.91a
NH_4^+–N (mg kg^{-1})	25.30 ± 2.57	1.42 ± 0.05	1.42 ± 0.13	1.40 ± 0.09	1.43 ± 0.08	1.58 ± 0.13a	1.15 ± 0.09b	1.50 ± 0.09a	1.64 ± 0.19a	1.56 ± 0.24ab
NO_3^-–N (mg kg^{-1})	0.23 ± 0.003	7.75 ± 0.29	8.73 ± 0.58a	6.83 ± 0.48b	7.86 ± 0.44ab	8.47 ± 0.73a	8.40 ± 0.52a	7.06 ± 0.46b	6.59 ± 0.71b	7.21 ± 1.12ab

Values are means ± standard errors. Different letters in the same row indicate significant differences at $P < 0.05$ among the treatments (different urbanization levels or different land uses). Low, low urbanization level; Moderate, moderate urbanization level; High, high urbanization level. Gre, green land; Agr, agricultural land; Res, residential land; Tra, transport land; Pub, public land; TC, total carbon; TN, total nitrogen; C:N, ratio of carbon to nitrogen.

Tra (Fig. 3a). Moreover, we found that high urbanization significantly increased soil methanotrophic abundance compared with low and moderate urbanization (Fig. 3b). Under low urbanization level, we found that Agr had the highest methanotrophic abundance, which was significantly higher than that under Gre; Tra and Pub had similar methanotrophic abundances (Fig. 3c). Under moderate urbanization level, Pub and Agr had significantly higher methanotrophic abundances than Gre, while Tra and Res had similar methanotrophic abundance (Fig. 3d). Under high urbanization level, Tra and Res had significantly higher methanotrophic abundance than Gre (Fig. 3e).

3.4. Key factors driving soil CH_4 uptake under urbanization

In general, potential soil CH_4 uptake was negatively correlated with urbanization degree, soil moisture content, C:N ratios and NO_3^-–N contents, whereas potential soil CH_4 uptake were positively correlated with NH_4^+–N contents across land uses (Table 2). However, we did not find a significantly positive correlation between potential soil CH_4 uptake and methanotrophic abundance across land uses (Table 2).

Furthermore, we used structural equation modeling to quantify the relative contributions of the degree of urbanization, methanotrophic abundance and soil properties to soil CH_4 uptake across urbanization levels. This SEM analysis represented the best fit to our data. In general, the model explained 12% of the variation in potential soil CH_4 uptake across all sites, and explained 21%, 39%, 15%, 22% and 66% in Gre, Agr, Res, Tra and Pub, respectively (Fig. 4). In general, potential soil CH_4 uptake were significantly influenced by urbanization degree and soil properties, with urbanization degree explaining 72% of the variance of potential soil CH_4 uptake across the sites (Fig. 4a). To our surprise, we did not find that methanotrophic abundance had an effect on potential soil CH_4 uptake across land uses, but they explained 5–34% of the variance of potential soil CH_4 uptake across land uses (Fig. 4b-4f). We found that potential soil CH_4 uptake was directly influenced by urbanization degree in Res, Tra and Pub, and was influenced by soil properties across all land uses except for Tra (Fig. 4b-4f).

Moreover, we found that potential soil CH_4 uptake was significantly influenced by soil properties under low urbanization level, but by soil properties and urbanization degree under moderate and high urbanization levels (Fig. 5). We also found that urbanization degree also significantly influenced potential soil CH_4 uptake via indirect effects on soil properties under low and high urbanization level. Similar to the results across land uses, we did not find a significant effect of methanotrophic abundance on potential soil CH_4 uptake under any urbanization level (Fig. 5). Finally, we combined all the data together and created a schematic graph to show the changes in potential soil CH_4 uptake and the associated methanotrophic abundance across urbanization levels (Fig. 6).

4. Discussion

In this study, we uncover the effects of urbanization on soil CH_4 uptake and the underlying microbial mechanisms. First, we acknowledge that CH_4 efflux measured in this study is the net effect of the processes of CH_4 production and CH_4 oxidation in the soils. However, many studies have proven that in well-aerated soils, CH_4 production rates are negligible, so CH_4 oxidation rates can be used to represent CH_4 uptake (Bu et al., 2019; Gu et al., 2019; Zhou et al., 2021). Second, to the best of our knowledge, our work is the first to quantify the effects of urbanization degree on soil CH_4 uptake. Our results provide clear evidence that urbanization significantly decreases soil CH_4 uptake via direct and indirect pathways. Moreover, urbanization can decouple the relationships between soil CH_4 uptake and methanotrophic abundance across urbanization levels (Nazaries et al., 2011; Kou et al., 2017).

Table 2
Pearson correlations of potential soil CH_4 uptake with urbanization degree, methanotrophic abundance and soil physicochemical properties under different land uses along a gradient of urbanization in Shanghai.

Sites	U	*pmoA*	Moisture	pH	TC	TN	C:N	NH_4^+–N	NO_3^-–N
All	−0.24**	−0.02	−0.15**	−0.07	−0.09	−0.05	−0.14*	0.19**	−0.13*
Gre	−0.33**	0.18	0.04	−0.13	0.02	0.09	−0.08	0.13	−0.41**
Agr	−0.11	0.21	−0.27**	0.04	−0.12	−0.14	0.02	0.21*	0.01
Res	−0.36**	−0.09	−0.17	−0.21*	−0.01	0.01	−0.001	0.07	−0.16
Tra	−0.39*	−0.06	−0.21	−0.003	−0.17	−0.10	−0.13	0.23	0.17
Pub	−0.54**	0.15	−0.75**	−0.22	−0.82*	−0.61*	−0.54**	0.59**	−0.05

Significance level: * $P < 0.05$; ** $P < 0.01$.
Gre, green land; Agr, agricultural land; Res, residential land; Tra, transport land; Pub, public land.
U, urbanization degree; TC, total C; TN, total N; C:N, ratio of carbon to nitrogen.

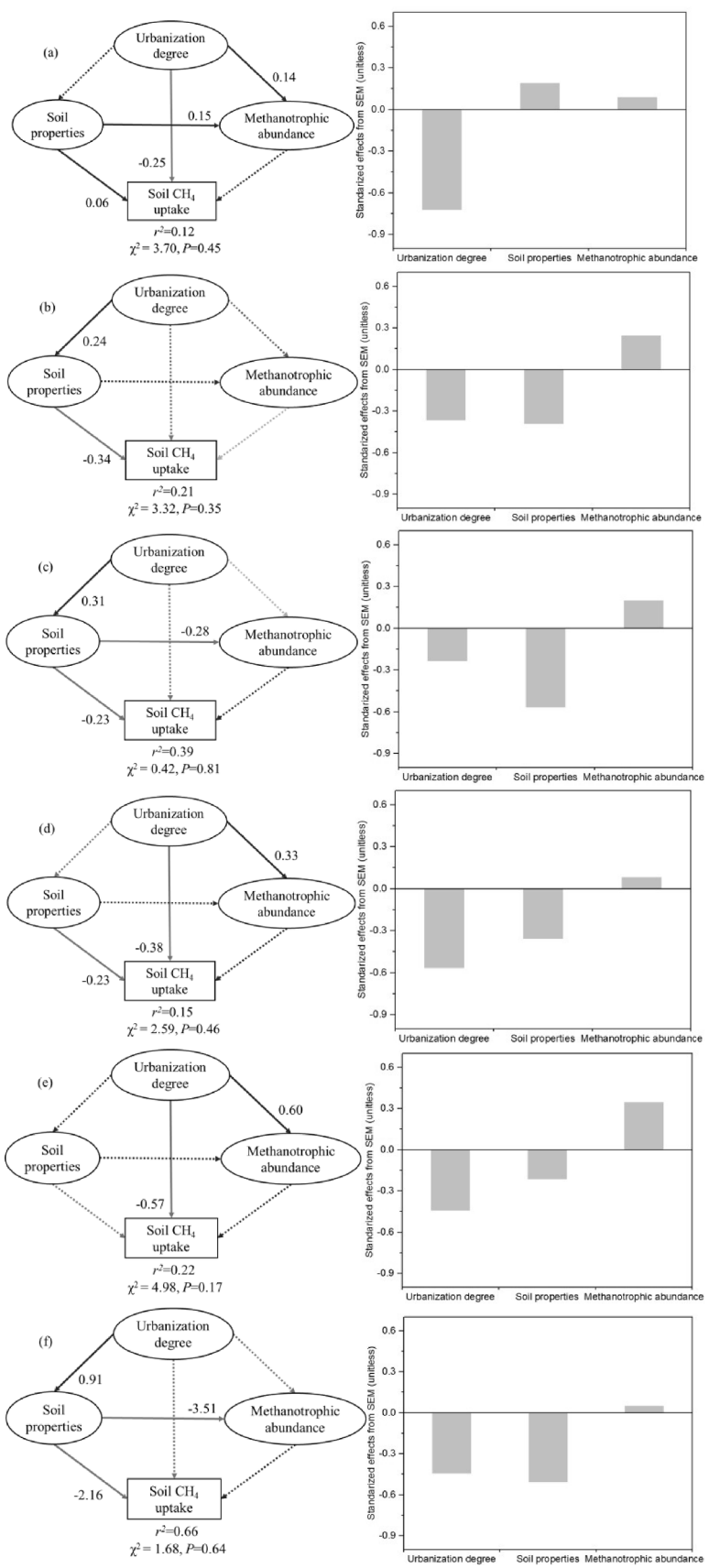

Fig. 4. Path diagrams representing the final model showing the contributions of urbanization degree, methanotrophic abundance and soil properties to potential soil CH_4 uptake across all sampling sites (a) and in green land (b), agricultural land (c), residential land (d), transport land (e) and public land (f) along a gradient of urbanization in Shanghai. The methanotrophic abundance is represented by *pmoA* gene copy numbers. The dotted line indicates no significance. The right-hand column shows the corresponding standardized total effects (direct plus indirect effects) derived from the structural equation model. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

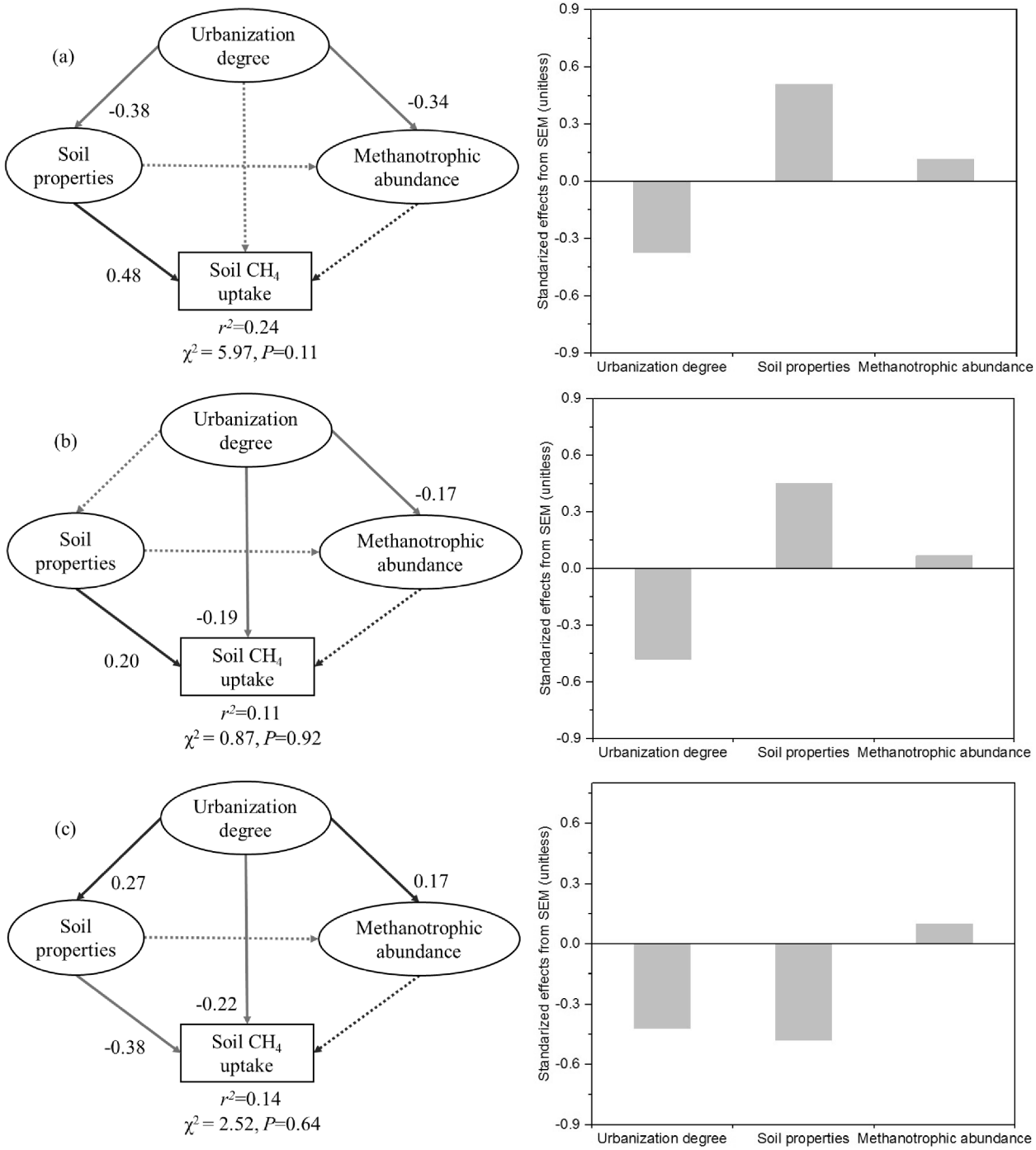

Fig. 5. Path diagrams representing the final model showing the contributions of urbanization degree, methanotrophic abundance and soil properties to potential soil CH_4 uptake under low (a) moderate (b) and high (c) urbanization level. The methanotrophic abundance is represented by *pmoA* gene copy numbers. The dotted line indicates no significance. The right-hand column shows the corresponding standardized total effects (direct plus indirect effects) derived from the structural equation model.

4.1. *Effect of urbanization on soil CH_4 uptake*

Increasing population densities and urban sprawl have been causing rapid land use changes from natural ecosystems into smaller residential and related recreational lands and so on (United Nations, 2014), which can greatly influence soil CH_4 uptake (Tate, 2015; van Delden et al., 2018). Potential soil CH_4 uptake has been widely used to represent soil CH_4 uptake in terrestrial ecosystems (Kou et al., 2017; Feng et al., 2020). Some studies have reported that potential soil CH_4 uptake can be used as an indicator of CH_4 uptake (Tate, 2015; Bu et al., 2019; Gu et al., 2019). Most previous studies have focused on differences in the efflux of CH_4 and other greenhouse gases between rural forests and urban turfgrass and lawns (Groffman and Pouyat, 2009; van Delden et al., 2018). However, little information is available about the effects of urban-related land use changes on soil CH_4 uptake.

In this study, we analyzed the effects of urbanization on soil CH_4 uptake worldwide and found lower soil CH_4 uptake under urbanization (0.83 ± 0.12 mg m^{-2} day^{-1} vs 1.17 ± 0.14 mg m^{-2} day^{-1}) (Fig. 1). Land use changes significantly decreased soil CH_4 uptake as well, with greater inhibition effects seen under higher urbanization level (Fig. 3). Notably,

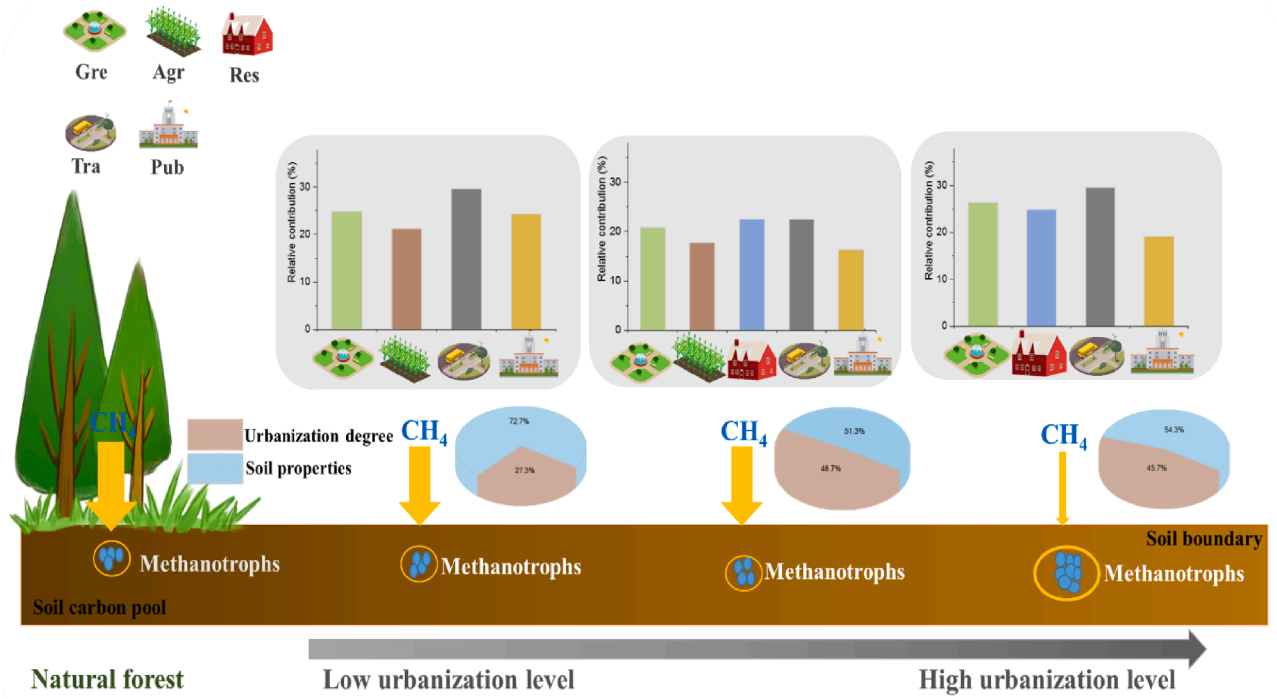

Fig. 6. Schematic diagram showing changes in soil CH_4 oxidation capacity as well as methanotrophic abundance in soils of an adjacent natural forest and along a gradient of urbanization in Shanghai. The changes in the soil carbon (C) pools are indicated by the soil color: a lighter color indicates lower soil C contents; a darker color indicates higher soil C contents. The width of the arrows indicates the strength of soil methanotrophic activity (the relative contribution of each land use is expressed by the percentage of soil CH_4 oxidation) and the number of blue circles indicates methanotrophic abundance. The pie charts indicate the relative contribution of urbanization degree and soil properties to soil CH_4 oxidation capacity along a gradient of urbanization. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

among the different land uses, Tra and Gre had relatively higher potential soil CH_4 uptake, which could be ascribed to tree plantations in these areas. Previous studies have demonstrated that plant growth can enhance the diffusion of atmospheric oxygen into the soil, thus increasing soil CH_4 oxidation (Kolb, 2009; Shukla et al., 2013). On the other hand, Pub and Agr had relatively lower potential soil CH_4 uptake (Fig. 3). The reason for this was the large amount of human activities such as N fertilizer applications. Many studies have shown that soil N acts as an important nutrient element, which can hamper soil CH_4 uptake at high N concentrations, although it can increase soil CH_4 oxidation at lower N concentrations (Kolb, 2009; Tate, 2015). These findings were supported by the higher soil NO_3^-–N concentrations in Pub and Agr (Table 1).

4.2. The key drivers influencing soil CH_4 uptake under urbanization

It is well known that soil CH_4 uptake is affected by many factors, including soil properties and methanotrophic communities (Tate, 2015). Previous studies have reported that the key drivers of soil CH_4 uptake are soil moisture contents, soil N availability, plant growth and so on (Kolb, 2009; Shukla et al., 2013; Tate, 2015). However, until now, no study has quantified the effects of urbanization degree on soil CH_4 uptake.

Urbanization can influence soil CH_4 uptake via direct and indirect pathways (Fig. 4). We acknowledge that urbanization can, to some extent, have a positive effect on soil CH_4 uptake (Grimm et al., 2008). For example, high urbanization levels can directly increase the atmospheric temperature, forming heat islands in urban regions (Oke and Maxwell, 1975). Given that high temperatures tend to promote the associated enzymatic processes of methanotrophic activity (Tate, 2015), heat islands can enhance soil CH_4 uptake. In addition, a previous study showed that urban regions had higher atmospheric CH_4 concentrations than rural regions, which may be helpful for increasing soil CH_4 uptake as well, because atmospheric CH_4 acts as a substrate for methanotrophs (Liu et al., 2019). In contrast with what we expected, however, our study clearly demonstrated that urbanization significantly decreased soil CH_4 uptake (Fig. 1 and Fig. 3), indicating that the negative effects of urbanization override the positive effects on soil CH_4 uptake. As we know, urbanization-derived land use changes, atmospheric pollution and large applications of fertilizer can hamper methanotrophic activity, thus decreasing soil CH_4 uptake (Grimm et al., 2008; Groffman and Pouyat, 2009; Shukla et al., 2013). All these may contribute to the strong inhibitory effects of high urbanization levels on soil CH_4 uptake (Fig. 5).

In addition, we did not find consistent changes in potential soil CH_4 uptake and methanotrophic abundance along a gradient of urbanization (Fig. 3, Fig. S1). Many studies have reported that methanotrophic abundance can be used to predict the changes in soil CH_4 uptake (Nazaries et al., 2011; Tate, 2015; Gu et al., 2019). In contrast, we found that urbanization increased methanotrophic abundance, with higher numbers under higher urbanization levels. Overall, our results indicated that urbanization decoupled the relationships between soil CH_4 oxidation and methanotrophic abundance, which is often neglected in current studies (Groffman and Pouyat, 2009; van Delden et al., 2018). We need to note that here we only targeted high-affinity methanotrophs (Gu et al., 2019; Lafuente et al., 2019), which could partly explain for the uncoupled relationship between CH_4 uptake and the abundance of methanotrophs under different urbanization levels.

5. Conclusions

Our results have clearly demonstrated that urbanization decreased soil CH_4 uptake worldwide, with greater inhibition effects under higher urbanization level. Different land use changes can significantly decrease soil CH_4 uptake as well. Among land uses, Tra and Gre had relatively higher soil CH_4 uptake, whereas Pub and Agr had relatively lower soil CH_4 uptake. Urbanization can influence soil CH_4 uptake via direct and

indirect pathways; the former had greater negative effects on soil CH_4 uptake than the latter. More importantly, urbanization can decouple the relationships between soil CH_4 uptake and methanotrophic communities, which is in contrast to the findings in natural ecosystems. To our knowledge, this is the first study to quantify the effects of urbanization degree on soil CH_4 uptake. Urbanization degree plays a predominant role in driving soil CH_4 uptake, which implies that urbanization degree should be considered in future Earth system models to improve understanding of soil CH_4 uptake in urban regions worldwide.

Declaration of Competing Interest

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

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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.2021.115368.

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