



## Afforestation enhanced soil CH<sub>4</sub> uptake rate in subtropical China: Evidence from carbon stable isotope experiments

Junjun Wu<sup>a</sup>, Qianxi Li<sup>a</sup>, Jingwen Chen<sup>a,b</sup>, Yao Lei<sup>a</sup>, Qian Zhang<sup>a,b</sup>, Fan Yang<sup>a,b</sup>, Dandan Zhang<sup>a,b</sup>, Quanfa Zhang<sup>a</sup>, Xiaoli Cheng<sup>a,\*</sup>

<sup>a</sup> Key Laboratory of Aquatic Botany and Watershed Ecology, Wuhan Botanical Garden, Chinese Academy of Sciences (CAS), Wuhan, 430074, PR China

<sup>b</sup> Graduate University of Chinese Academy of Sciences, Beijing, 10039, PR China

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### ABSTRACT

Afforestation plays an important role in regulating the methane (CH<sub>4</sub>) exchange between soil and atmosphere. However, it is not fully understood how afforestation affects soil CH<sub>4</sub> flux and the carbon isotopic signature of CH<sub>4</sub>. We conducted a year-long measurement of CH<sub>4</sub> in afforested land (woodland and shrubland) and the adjacent cropland using the static chamber-gas chromatographic technique in the Danjiangkou Reservoir of central China. The soil exclusively functioned as a sink for atmospheric CH<sub>4</sub> through the entire study period across land use types. Land use types significantly impacted the CH<sub>4</sub> uptake rate with the largest average CH<sub>4</sub> uptake rate in the shrubland (37.22 μg m<sup>-2</sup>h<sup>-1</sup>), followed by the woodland (27.75 μg m<sup>-2</sup>h<sup>-1</sup>) and the cropland (14.34 μg m<sup>-2</sup>h<sup>-1</sup>). The mean annual CH<sub>4</sub> uptake rates increased in the shrubland by 186.3% and the woodland by 93.5%, compared to the cropland. The isotope fractionation factor (α<sub>soil</sub>) was lower in the woodland and shrubland, compared to the cropland. The CH<sub>4</sub> uptake rates and α<sub>soil</sub> exhibited similar seasonal patterns among land use types, with a higher CH<sub>4</sub> uptake rates and lower α<sub>soil</sub> in spring and summer compared to other seasons. The CH<sub>4</sub> uptake rates were positively related to microbial biomass carbon (MBC) and labile C. Meanwhile, the CH<sub>4</sub> uptake rate was exponentially correlated with inorganic nitrogen (N) concentration, suggesting the high inorganic N concentration in the cropland possibly inhibited the CH<sub>4</sub> uptake rate. In afforested land, CH<sub>4</sub> uptake rates positively correlated with soil temperature and negatively correlated with the C:N ratio. The α<sub>soil</sub> was negatively related to soil temperature, whereas the δ<sup>13</sup>C values of CH<sub>4</sub> remaining in the chambers were positively related to the δ<sup>13</sup>C values of soil organic carbon (SOC) and MBC. Our results suggest that the change in soil properties (i.e. high SOC and MBC, low C:N ratio and low inorganic N) following afforestation is a critical control on enhanced CH<sub>4</sub> uptake capacity, while a lower α<sub>soil</sub> further provides evidence for a high CH<sub>4</sub> uptake rate in afforested lands.

### 1. Introduction

Methane (CH<sub>4</sub>), the second most important greenhouse gas, plays an important role in the chemical and radiative balances in the Earth's atmosphere (Ghosh et al., 2015), and its global warming potential (GWP) is 28–34 times higher than that of CO<sub>2</sub> on a 100-year timescale (IPCC, 2013), which accounts for up to 20–30% of global warming effect (IPCC, 2007). It has been suggested that the aerobic soil is the second largest global sink for atmospheric CH<sub>4</sub>, estimated to be of 20–45 Tg yr<sup>-1</sup> (Dutaur and Verchot, 2007; IPCC, 2013), accounts for up to 5–10% of the global CH<sub>4</sub> sink (Aronson et al., 2013a). Among the various ecosystems, forest soils have been identified as the most efficient sinks (Kolb, 2009) with CH<sub>4</sub> sink strength following this order: woodland > no-cultivated upland > grassland > cultivated soils

(Levine et al., 2011; Nazaries et al., 2013a). However, human activities, such as deforestation and conventional tillage can shift the balance between soils acting as a CH<sub>4</sub> source or a sink (Tate, 2015; Baah-Acheamfour et al., 2016; Chamberlain et al., 2017).

Soil CH<sub>4</sub> flux in terrestrial systems is sensitive to land use change and it can greatly alter CH<sub>4</sub> consumption by changing soil chemical and physical properties as well as microbial activities (Nazaries et al., 2013b; Singh and Gupta, 2016). Numerous studies have indicated that cropland can uptake less CH<sub>4</sub> than grassland and forest due to tillage and nitrogen fertilization (Aronson and Helliker, 2010; Plaza-Bonilla et al., 2014; Zhang et al., 2017). Conversely, Rong et al. (2015) have reported that cropland has higher CH<sub>4</sub> uptake than grassland because of the decrease in soil bulk density. Other studies have indicated that bio-based residue application on agriculture land can stimulate the

\* Corresponding author.

E-mail address: [xlcheng@fudan.edu.cn](mailto:xlcheng@fudan.edu.cn) (X. Cheng).

methane oxidation rate, leading to higher soil methane uptake than in natural soils (Ho et al., 2015). Generally when plant effects are observed, it is plant functional type differences that are of interest, with the soil around trees associated with higher CH<sub>4</sub> consumption than shrubs and grasses (Tate et al., 2007; Aronson et al., 2013a). Saggar et al. (2008) and Hiltbrunner et al. (2012) have found that soil CH<sub>4</sub> oxidation substantially increases when established pastures have been planted in pine (*Pinus radiata*), and Norway spruce, respectively, and this is initially attributed to a decrease in soil moisture under the trees. Additionally, plant species can also influence the CH<sub>4</sub> uptake capacity of soils. For instance, soils in broadleaf deciduous forests have been found to have higher CH<sub>4</sub> uptake rates than that in coniferous forests (Degelmann et al., 2010; Aronson et al., 2013a), which is likely related to pH and the chemical compounds released by plants (Maurer et al., 2008; Degelmann et al., 2010).

Isotopic signature of the CH<sub>4</sub> can provide an important constraint for modeling sources and sinks in the global CH<sub>4</sub> budget and give information about the exploration of CH<sub>4</sub> production and oxidation processes in the terrestrial ecosystem (Moeller et al., 2013; Vaughn et al., 2016). Methanogenesis produces methane depleted in <sup>13</sup>C, whereas microbial oxidation (methanotrophy) strongly fractionates to cause enrichment in <sup>13</sup>C of the remaining methane (Cadieux et al., 2016; Fisher et al., 2017). This is because the oxidation of CH<sub>4</sub> is always accompanied with kinetic isotope effect (KIE), which means <sup>12</sup>C-CH<sub>4</sub> is preferentially removed during its oxidative consumption and the heavier <sup>13</sup>C-CH<sub>4</sub> is left (Whiticar, 1999). The KIE associated with microbial oxidation of atmospheric CH<sub>4</sub> has been found primarily determined by physical properties that impact gas diffusion, as well as the activity of high-affinity methanotrophs (Maxfield et al., 2008). Thus, stable isotope of CH<sub>4</sub> is an indicator to quantify the CH<sub>4</sub> flux dynamics when afforestation results in a shift in the physical and biological properties of soil environment.

The Danjiangkou Reservoir, established in the 1970s, with a drainage area of 95,200 km<sup>2</sup>, is a water source area of China's Middle Route of South-to-North Water Transfer Project (Zhang et al., 2009). Human activities such as conventional tillage and deforestation around the reservoir have resulted in numerous environmental problems, such as soil erosion, water pollution and soil C and N losses. In recent decades, afforestation has been conducted to restore and protect the Danjiangkou Reservoir riparian ecosystem. Previous works in this region has demonstrated that afforestation could enhance soil C sequestration (Cheng et al., 2013), and also soil respiration (Dou et al., 2016). However, little is known about how afforestation affects the role of soils acting as CH<sub>4</sub> sink in this area. Here, our overall objective was to assess the effect of afforestation on CH<sub>4</sub> uptake rate and its carbon-isotopic signature. We would have two predictions. First, we predicted that the afforestation would improve the CH<sub>4</sub> uptake capacity of soils and alter carbon isotopic signature of the CH<sub>4</sub>. Second, we also predicted that plant species (tree and shrub) would have different influence on the CH<sub>4</sub> uptake capacity of soils. To test these hypotheses, we conducted a year-long measurement of CH<sub>4</sub> in afforested (woodland and shrubland) and the adjacent croplands. We also investigated the microclimate, soil properties and microbial biomass to explore potential control on the CH<sub>4</sub> uptake capacity of soils and carbon isotopic signature of the CH<sub>4</sub>.

## 2. Materials and methods

### 2.1. Study site

This study was conducted at the Wulongchi Experiment Station (32°45'N, 111°13'E; 280–400 m a.s.l.) in the Danjiangkou Reservoir area. The climate in this area belongs to the subtropical monsoon of the north subtropical zone. The mean annual temperature is 15.7 °C, with monthly averages of 27.3 °C in July and 4.2 °C in January. The annual precipitation is 749.3 mm, of which 70–80% occurs between April and October. The soil is yellow-brown with 11% sand, 41% silt, and 48%

clay in the top 30 cm. In 1980s, following a reorganization of the land use, a large uncultivated area was converted to a woodland plantation, with coniferous plants (*Platycladus orientalis* (Linn.) Franco), and a shrubland plantation (*Sophora davidii*) (Zhu et al., 2010). Based on our surveys, there were 53 trees per 100 m<sup>2</sup> in woodland plantation, and the average DBH (diameter at breast height) is 6.4 cm. The basal diameter of *Sophora davidii* in shrubland is always less than 2 cm. Farmers typically cultivated corn and rape in cropland. Corn and rape cultivation was managed by conventional agricultural practices including plowing to a 0.4 m depth, mineral fertilizations (approximately urea 375 kg ha<sup>-1</sup> and urine ammonium 200 kg ha<sup>-1</sup>) and chemical weeding. The aboveground biomass of corn and rape was removed through harvesting.

### 2.2. Experimental design and CH<sub>4</sub> flux measurement

The experimental design was a randomized block design. We selected four blocks which contains three land types-woodland, shrubland, and adjacent cropland (i.e. the control). The distance between each block was around 50 m. Each block was approximately 2000 m<sup>2</sup> (20 m × 100 m). Within each block, three sub-sites (5 m × 5 m) were randomly selected in plant rhizosphere (the place around root about 1–1.5 m) for the three land use types, respectively. The CH<sub>4</sub> flux was measured twelve times from March 2014 to March 2015 (once every month) using static chambers and the gas chromatography technique. Two PVC collars (30 cm diameter, 15 cm height, 5 mm thickness) were inserted 10 cm into the soil of each sub-site. A removable top lid (30 cm diameter, 30 cm height, 5 mm thickness) on which had a gasket to ensure air-tightness during sampling. On the top wall of each chamber cover, a battery-operated fan of 10 cm diameter was installed to mix the air in the chamber while the sample was collected. There was a hole located on the top wall of the upper chamber, and the hole was connected with a 12 cm long silicon tube (5 mm in diameter), which was used for air collection. It was maintain leak tight by a bulldog clip during the incubation period.

Methane fluxes were calculated from measurement of the methane mole fraction change in the enclosed headspace. Four samples were collected by syringe throughout a 45 min incubation period (at 0, 15, 30, and 45 min) and transferred to 20 mL pre-evacuated tedlar (aluminium foil compound membrane, Delin gas packing Co., Ltd, Dalian, China) bags. Simultaneously, the air temperature of each experimental plot was measured with a mercurial thermometer. Soil temperature and moisture were measured outside each chamber with a portable instrument that measured soil temperature and moisture (SIN-TH8, SinoMeasure, China). CH<sub>4</sub> concentrations of the gas samples stored in tedlar bags were measured with a flame ionization detector (FID) in a gas chromatography (Agilent 7890B, Santa Clara, CA, USA).

The CH<sub>4</sub> fluxes were calculated using linear model regression analysis of the change in gas concentration in the chambers with time over a 45-min period with an average chamber temperature (Zhang et al., 2008):

$$F = \frac{dc}{dt} \times \frac{16}{22.4} \times \frac{273}{273 + T} \times \frac{V}{A} \quad (1)$$

where F is the CH<sub>4</sub> flux (μg·m<sup>-2</sup>·h<sup>-1</sup>),  $\frac{dc}{dt}$  is the rate of change in gas concentration inside the chamber, T is the air temperature inside chambers, 16 is the molecular weight of CH<sub>4</sub>, 22.4 is the molar volume of an ideal gas at standard temperature and pressure (1 mol<sup>-1</sup>), V is the chamber volume (m<sup>3</sup>) and A is the chamber area (m<sup>2</sup>). CH<sub>4</sub> fluxes were discharged if the regression coefficients (r<sup>2</sup>) were below 0.9.

Samples of air were also collected from each chamber for isotopic analysis. The chambers were closed for 45 min before a sample of air was collected from the chambers. The inlet of a gas pump operated by a 6 V battery was connected to a three-way valve on the chamber using flexible tubing. The valve to the chamber was opened, and the pump was run for 2 s to flush the tubing in the line with air from the chamber.

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