

**Tree stem and soil methane
fluxes in temperate, upland
forests: sources, sinks & drivers**

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Abstract

Methane (CH₄) and carbon dioxide (CO₂) are two important greenhouse gases (GHG). Mineral soils in temperate forests are one of the most significant biological sinks for CH₄. However, recent studies reported tree stem CH₄ emissions may offset soil CH₄ sink and contribute to the global CH₄ budget. Current knowledge is still limited on the pathway and underlying mechanism of stem CH₄ emissions and uptake. Therefore, this thesis investigated the exchange of CH₄ and CO₂ fluxes from tree stems and soils in temperate upland forests.

The presence of ECM mycelium exhibited a fluctuation effect on soil CH₄ uptake and soil respiration during the short-term 1-2 years, but significantly decreased the cumulative CH₄ uptake during the long-term 8.5-9.5 years. Biochar addition did not have any significant effect on soil CH₄ uptake and mostly no effect on soil respiration over the entire study.

Tree stem CH₄ flux did not show any significant differences between English oak (*Quercus robur*) and Japanese larch (*Larix kaempferi*) trees during a growing season. The seasonal pattern of stem CH₄ flux was not found, but large daytime and intra-specific variations in oak and larch stem CH₄ fluxes were observed showing both CH₄ uptake and emission.

High-frequency measurements (1.5 hourly frequency) of three white poplar (*Populus alba*) tree stem CH₄ and CO₂ fluxes showed large hour-to-hour variation in stem CH₄ flux with both CH₄ uptake and emissions, and a clear diurnal pattern of stem CO₂ flux with larger flux during night-time than daytime. Wood incubation experiments showed the highest rates of potential CH₄ production and CH₄ oxidation were from bark. Combined with the results of lower internal stem CH₄ concentration and soil as a net CH₄ sink, it suggests that biologically *in situ* tree stem CH₄ production is the major source of stem CH₄ emission in temperate upland forests.

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Two roads diverged in a wood, and I

I took the one less traveled by,

And that has made all the difference.

By Robert Frost, The Road Not Taken

Declaration

I declare that this thesis is a presentation of original work and I am the sole author. This work has not previously been presented for an award at this, or any other, University. All sources are acknowledged as References.

1. Introduction

1.1 Background of CH₄ in forests

Methane (CH₄) is the second most important greenhouse gas (GHG) after carbon dioxide (CO₂), contributing more than 20% of total anthropogenic GHG emissions (Pratt and Tate, 2018). Atmospheric CH₄ concentration has risen from about 722 ppb at the pre-industrial level in 1750 to 1910.8 ppb in 2021 (Dlugokencky, December 2021). Methane has a shorter lifetime compared with CO₂, so the reduction of CH₄ emissions will be an effective pathway for rapidly decreasing the rate of climate warming (Tian et al., 2016). It is estimated that tropospheric CH₄ lifetime is 9.3 years and the total CH₄ lifetime is 8.2 ± 0.8 years (Saunois et al., 2016). Although CH₄ has 28 times larger global warming potential (over a 100-year time horizon) (GWP₁₀₀) than CO₂ (IPCC, 2013), from 1750 to 2011 radiative forcing (RF) for CH₄ (0.48 ± 0.05 W m⁻²) is around three times lower than CO₂ (1.82 ± 0.19 W m⁻²) (Myhre et al., 2013). More recent estimates (Etminan et al., 2016) showed CH₄ RF is about 25% higher from 1750 to 2011 than the value in the Intergovernmental Panel on Climate Change (IPCC) 2013 assessment and the global warming potential on centennial time scales is 14% higher than the IPCC value. Using GWP₁₀₀ reported by the IPCC Sixth Assessment Report (AR6) (Forster et al., 2021), the global GHG emissions in 2018 reached 58 ± 6.1 GtCO₂eq, of which CH₄ emissions contributed 17.24% (Minx et al., 2021).

1.1.1 Global methane budget

Measurements of atmospheric CH₄ on a global scale showed a persistent increase in the 1980s and a slowdown in growth in the 1990s, followed by little change from 1999 to 2006. Since 2007, atmospheric CH₄ levels have been rising again and the increase depends on the imbalance between CH₄ sources and sinks (Rigby et al., 2008; Kirschke et al., 2013). Global CH₄ emissions were estimated by top-down inversions at 576 Tg CH₄ yr⁻¹ between 2008 and 2017 and approximately 60% (range 50-65%) of CH₄ comes from anthropogenic sources including agriculture and waste, fossil fuel exploitation, and biomass burning (Saunois et al., 2020). Natural sources such as natural wetlands, freshwater, wild animals, termites, geological, permafrost and hydrates are responsible for the rest of the global CH₄ budget. The top-down estimate of CH₄ emissions from natural sources was 218 Tg CH₄ yr⁻¹ at a global scale

during the 2000s, of which natural wetlands accounted for approximately 80% (Kirschke et al., 2013).

The atmospheric CH₄ sinks include CH₄ oxidation in the atmosphere (Dlugokencky et al., 2011; Saunio et al., 2016), the uptake of CH₄ by soils (Curry, 2007; Dutaur and Verchot, 2007) and the reaction with chlorine radicals (Cl) from sea salt in the marine boundary layer which only accounts for around 3% of the global CH₄ sink (Allan et al., 2007; Thornton et al., 2010; Kirschke et al., 2013). The oxidation of CH₄ by the hydroxyl radical (OH) is the main atmospheric sink, mostly taking place in the troposphere, and the bottom-up estimate of this sink is 528 Tg CH₄ yr⁻¹ with a large range (454 - 617) in the 2000s, accounting for around 90% of the global CH₄ sink (Kirschke et al., 2013). Around 3% of the global CH₄ is removed by reaction with different oxidants (excited atomic oxygen O (1D), atomic chlorine (Cl), atomic fluorine (F) and OH) in the stratosphere (Voulgarakis et al., 2013; Williams et al., 2012; Kirschke et al., 2013). Methane uptake by soils contributes to a relatively small net CH₄ sink, but it is a vital part of the global atmospheric CH₄ budget and the only terrestrial sink. Based on different models which involve various factors related to CH₄ consumption rate in soils, estimates of global CH₄ soil uptake are reported as a climatological range of 9–49 Tg CH₄ yr⁻¹ during 2000 to 2017, accounting for approximately 4% of the global CH₄ sink (Kirschke et al., 2013; Ciais et al., 2013; Saunio et al., 2016, 2020).

Forest soils are thought to be optimal for CH₄ consumption (Smith et al., 2000; Boeckx and Van Cleemput, 2001). It was reported that the estimate of global mean CH₄ uptake in forest soils (4.2 kg CH₄ ha⁻¹ yr⁻¹) was higher than those of other ecosystems (1.6 kg CH₄ ha⁻¹ yr⁻¹) (Dutaur and Verchot, 2007). More recent data also showed similar analysis of the global mean CH₄ uptake in forest soils during 1988 to 2015 (3.8 kg CH₄ ha⁻¹ yr⁻¹) (Ni and Groffman, 2018). The estimates of global CH₄ uptake in forest soils are summarized in Table 1.1. It was reported that CH₄ consumption in tropical forest soils was 5.79 Tg CH₄ yr⁻¹, contributing approximately 63% to the total uptake in global forest soils, followed by CH₄ uptake in temperate soils (2.43 Tg CH₄ yr⁻¹) and lowest in polar/boreal soils (0.95 Tg CH₄ yr⁻¹) (Yu et al., 2017). Some studies indicated that CH₄ uptake in forest soils increased during 1980-2009 (Curry, 2009; Hashimoto et al., 2011; Zhuang et al., 2013; Yu et al., 2017). However, recent research suggested that the current soil CH₄ sink may be overestimated over large regional areas, as CH₄ uptake in forest soils around the globe has decreased by an average of 77% from 1988 to 2015, especially

forests located from 0 to 60°N latitude, due to increases in precipitation and soil moisture content (Ni and Groffman, 2018).

Table 1. 1 Estimated global CH₄ uptake in forest soils (Yu et al., 2017)

References	Period	Resolution	Forest		
			Area (× 10 ⁶ km ²)	Flux (kg CH ₄ ha ⁻¹ yr ⁻¹)	Uptake (Tg CH ₄ yr ⁻¹)
Dutaur and Verchot (2007)	NA	NA	42.0	2.76	11.6
Curry (2007)	1979-1999	3.75° × 3.75°	73.9	2.46	18.15
Yu et al. (2017)	1981-2000	0.5° × 0.5°	42.0	2.18	9.15

NA, not available

1.2 Methane processes in soils

Net CH₄ exchange between soils and the atmosphere depends on the net balance of two contrasting microbial processes – CH₄ production and CH₄ oxidation. Soils are considered as sinks for atmospheric CH₄ when the activities of methane oxidizers (methanotrophs) generally dominate over those of methane-producing archaea (methanogens) (Conrad, 2009). Methanotrophs which use CH₄ as a sole source of carbon (C) and energy are able to oxidize atmospheric CH₄ in drier soils (Hanson and Hanson, 1996; Denman et al., 2007). There are two different modes of net CH₄ uptake between soils and the atmosphere. High-affinity methanotrophs can consume CH₄ at atmospheric concentration (~1.8 ppm), which primarily takes place in aerobic soils. While low-affinity CH₄ oxidation is performed by methanotrophs that act as biofilters at oxic-anoxic interfaces in high CH₄ flux environments (>100 ppm) (Serrano-Silva et al., 2014; Tate, 2015). Methane production in soils is attributed to methanogens in strictly anaerobic conditions at a very low redox potential, which is the terminal step of the anaerobic degradation of organic matter (Conrad, 1989). Methanogens can use acetate, formate, hydrogen (H₂) and CO₂ fermented by other anaerobes as major substrates for methanogenesis (Nazaries et al, 2013). Methane is usually produced by acetoclastic methanogenesis (using acetate as substrate) and hydrogenotrophic methanogenesis (using H₂/CO₂ as substrate) in anoxic soils (Schink and Stams, 2013; Liu, Klose and Conrad, 2019). Although theoretically acetoclastic methanogenesis accounts for more than 67% of CH₄ production (Conrad, 1999), acetoclastic methanogenesis and hydrogenotrophic methanogenesis shifted along with the bacterial and archaeal community structures in different soil types and at various temperatures (Metje and Frenzel, 2007; Liu,

Klose and Conrad, 2019). It has been shown that the structure and function of the methanogenic microbial community drastically changed at thermophilic (45°C) temperature compared to mesophilic (25 and 35°C) temperature (Liu, Klose and Conrad, 2019). Methane can be emitted into the atmosphere through diffusion, ebullition or plant-mediated transport (Smith et al., 2003; Serrano-Silva et al., 2014). Diffusion is a purely physical and slow process of CH₄ emission due to the low solubility of CH₄ in water (Neue, 1993), while CH₄ transported in the form of bubbles through steady or episodic ebullition often takes place when the production of CH₄ is high (Green, 2013). Plant-mediated CH₄ transport from the rhizosphere to the atmosphere through aerenchymatous tissue of plants adapted to waterlogged soil conditions, accounts for about 60-90% of CH₄ transportation to the atmosphere from the rice field and anoxic wetland environments (Shannon et al., 1996; Setyanto et al., 2004). Research areas with vascular plant (*Eriophorum vaginatum* L.) showed significantly higher CH₄ emissions than from similar areas without the plant serving as the direct conduits in an ombrotrophic peatland (Greenup et al., 2000).

At larger temporal and spatial scales, surfaces of the forests are commonly divided into wetlands (hydromorphic soils) and drylands (well-aerated or mineral soils), which are considered CH₄ sources and CH₄ sinks, respectively (Grunwald et al., 2012). Forest soils can act as both net CH₄ sinks and sources. It was reported that well-drained mineral forest soils are one of the most significant global biological sinks for CH₄ (Smith et al., 2000; Le Mer and Roger, 2001; Dutaur and Verchot, 2007), while hydromorphic forest soils in anaerobic conditions such as flooded forests are CH₄ sources (Smith et al., 2000; Butterbach-Bahl and Papen, 2002). Mineral soils which are usually considered sinks for atmospheric CH₄ can also support low rates of CH₄ production, thus methanotrophs may depend on two CH₄ sources, the atmosphere and the soil itself (Conrad, 1994; Chan and Parkin, 2001a). It was observed that forest soils harbour populations of methanogens and can emit CH₄ during wet periods and anaerobic conditions due to seasonal shifts in precipitation and evapotranspiration (Megonigal and Guenther, 2008; Dalal et al., 2008; Shrestha, Strahm and Sucre, 2015). However, methanogenesis has also been observed in well-drained mixed hardwood forest soils (Hudgens and Yavitt, 1997). Von Arnold et al. (2005a) found that deciduous forest soils in both drained and undrained sites were net emitters of CH₄, but the average annual CH₄ emission at the undrained site was almost ten times larger than the drained sites. Grunwald

et al. (2012) found that the forests of the cold climates and temperate zones are two important CH₄ sinks in Europe, with an annual uptake of 698 and 402 Gg CH₄ yr⁻¹, respectively. However, these zones may switch from being net CH₄ sinks to net CH₄ sources when wet forests were taken into consideration. Unlike other ecosystems, forest soils are generally seen as CH₄ sinks and their role as potential CH₄ sources in the global CH₄ budget is usually underestimated (Grunwald et al. 2012), which may explain the smaller estimation of carbon sink in forest ecosystems (-204 versus -363 Tg C m⁻² yr⁻¹) (Schulze et al., 2009).

1.3 Factors of CH₄ exchange in soils

Temperate forests account for 25% of the world's forests including large areas of North America, Europe, and Asia (Tyrrell, Ross and Kelty, 2012). It was reported that tree canopy has experienced the largest gain (+726,000 km², +33%) in temperate continental forests compared to other forest biomes from 1982 to 2016 (Song et al., 2018). According to the uncertainties of global soil CH₄ uptake ranging from 9 to 49 Tg CH₄ yr⁻¹ during 2000 to 2017 (Kirschke et al., 2013; Ciais et al., 2013; Saunio et al., 2016, 2020), which mainly comes from the great variation in climate and soils, therefore, attentions need to be drawn to temperate soil CH₄ exchange. There are two contrasting processes in CH₄ exchange, i.e. methanogenesis and methanotrophs, can be affected by soil organic matter, soil pH, soil moisture and texture, temperature, the concentration of O₂ and nitrogen (N) sources, biochar, and diurnal and seasonal variation (Dalal et al., 2008; Serrano-Silva et al., 2014; Tate, 2015; Malyan et al., 2016; Li et al., 2018). It was reported that tree species, atmospheric CH₄ concentration and mycorrhizal symbiosis in temperate forests can also affect CH₄ consumption (Menyailo and Hungate, 2003; Redeker, Baird and Teh, 2015; Subke et al., 2018).

1.3.1 Soil moisture

Soil moisture has been considered a major regulator of CH₄ exchange in soils, which is usually expressed as water-filled pore space (WFPS). CH₄ consumption rate can be reduced under conditions of high or low water content (Reay, Smith and Hewitt, 2007; Wei et al., 2018). For many soils 60% WFPS is approximately field capacity and it is reported that CH₄ uptake decreased with the soil moisture increasing from 60 to 100% WFPS due to gas transport limitation in a temperate forest in America (Castro et al., 1995). The optimum CH₄ uptake rates ranged diversely from 20% to 60% WFPS (Whalen and Reeburgh, 1996; Bowden,

Newkirk and Rullo, 1998; Khalil and Baggs, 2005; Borken et al., 2006; Reay, Smith and Hewitt, 2007; Schaufler et al., 2010). However, extremely low water contents can also diminish CH₄ oxidation rate by limiting the biological activity of methanotrophs (Khalil and Baggs, 2005; Borken et al., 2006; Wu et al., 2011). The water content is important for substrate supply for soil microorganisms (Schaufler et al., 2010) and higher soil moisture may influence CH₄ flux by limiting diffusion rates of the substrate to soil methanotrophs (Khalil and Baggs, 2005; Wang et al., 2013). An increase in soil moisture can decrease air-filled pore space and hence limit the diffusion of atmospheric CH₄ through the soil to methanotrophs, as molecular CH₄ diffusion in water is a factor 10⁴ slower than in air and thus soil CH₄ uptake is decreased (Bender and Conrad, 1995; Borken et al., 2006; Reay, Smith and Hewitt, 2007). It is indicated that soil moisture often has a negative correlation with CH₄ oxidation rate under most non-drought conditions (Whalen and Reeburgh, 1996; Khalil and Baggs, 2005; Borken et al., 2006; Schaufler et al., 2010; Subke et al., 2018).

Studies even have shown that forest soils can switch from a net CH₄ sink to a net CH₄ source when waterlogged (Schaufler et al., 2010; Grunwald et al., 2012, Yamulki et al., 2021). Methane is produced in soils by methanogens in strictly anaerobic conditions at a very low redox potential and such low redox conditions usually require prolonged waterlogging (Smith et al., 2003). Groundwater level can therefore also be a key driver as to whether forest soils emit or take up CH₄ (Conrad, 1989; Sundh et al., 1994; Granberg et al., 1997; Von Arnold et al., 2005a). Von Arnold et al. (2005a) found that soils in two drained sites of deciduous forests were both net CH₄ emitters and the difference in CH₄ emissions between the two sites was probably due to differences in their groundwater table levels. Lower depth of water table, which is closer to the soil surface can cause anaerobic conditions due to the low permeability of the soil. Several studies showed that CH₄ emission has a negative correlation with the depth of groundwater table in forest soils (Von Arnold et al., 2005b; Krause, Niklaus and Schleppe, 2013; Christiansen et al., 2016). Methane consumption rates are higher when soil water content is low and aerobic conditions prevail, whereas CH₄ production rates are higher when soil water content is high and anaerobic conditions may arise (Chan and Parkin, 2001b; Díaz et al., 2018). The rain simulation field experiments in a temperate forest in China showed that CH₄ uptake rates decreased with the intensity of wetting (Xu and Luo, 2012). Similar results were also found by Wu et al. (2011) in a temperate spruce forest soil, with CH₄ uptake

decreasing with increasing annual precipitation. Along similar lines, it was reported that prolonged summer droughts increased annual CH₄ uptake by soils in a spruce forest in Germany (Borken, Brumme and Xu, 2000), whilst Itoh, Ohte and Koba (2009) found that during heavy summer precipitation, the wetter site was a net CH₄ source compared to drier areas, which were net sinks of CH₄ in a temperate forest. Christiansen et al. (2016) found that the abundance of methanogens responded positively to an increase in soil moisture, which was significantly higher in wet forest soils than in mineral soils. And the ratio between relative abundances of methanotrophs and methanogens changed over the soil moisture gradient in a temperate rainforest, with the highest ratio in the mineral soils and close to 1 in the wet soils, which was related to net CH₄ exchange.

1.3.2 Temperature

There is contradictory information about the effect of temperature on CH₄ fluxes. Lab incubation experiments indicated that the effect of soil temperature on CH₄ oxidation is small, with reported Q₁₀ values (rate of reaction at t+10°C/rate of reaction at t, over 5° to 15°C) of the order of 1.4, which is mainly attributed to limited available CH₄ substrate (Smith et al., 2003). Additionally, it was reported that CH₄ consumption varied little within incubation temperatures from -1° to 30°C and the activity of methanotroph cultures did not show a response to temperature under conditions of phase-transfer limitation (when substrate consumption is phase transfer limited; Robinson and Tiedje, 1982) (King and Adamsen, 1992). Except for the frost period which gas diffusion is limited by ice in soil pores, many studies have shown that a lack of apparent temperature effect on CH₄ fluxes was found in temperate forest soils (Borken et al., 2006; Gundersen et al., 2012; Krause, Niklaus and Schleppei, 2013; Wang et al., 2013). Soil CH₄ uptake was positively correlated with wind speed and the lack of the temperature effect on soil CH₄ uptake may be due to the limited CH₄ substrate which might be caused by constant wind speed over the surface or sufficiently static measuring chamber systems (Redeker, Baird and Teh, 2015). Although some studies reported that CH₄ uptake was positively related to air temperature (Bradford et al., 2001; Yamulki and Morison, 2017) and soil temperature from 0° to 20°C (Ueyama et al., 2015; Yang, Wang and Xu, 2017) in temperate forest soils, no further explanations were provided. Studies have shown that CH₄ consumption rate was only influenced at low temperature due to its inhibition of microbial activity, suggesting that above a certain temperature threshold (10°C), CH₄ diffusivity may be

the main controlling factor of CH₄ uptake (Castro et al., 1995; Steinkamp, Butterbach-Bahl and Papen, 2001; Wu et al., 2011). Xu and Luo (2012) studied the sensitivity of temperature on CH₄ uptake in a temperate forest soil and found that temperature has a greater positive effect on CH₄ uptake under dry conditions than under wet conditions. It was suggested that temperature can only be a vital biological controller when soils were dry enough without diffusional limitation (Crill, 1991). Contrast to other findings, a recent study reported that net CH₄ uptake was negatively correlated with temperature ranging from 0° to 25°C in a temperate forest soil (Subke et al., 2018). A possible explanation is that as CH₄ is a poorly soluble hydrophobic compound and higher temperature may decrease CH₄ dissolution rates, thus suppressing the supply of aqueous-phase CH₄ to methanotrophs and reducing CH₄ uptake rate (Teh et al., 2006; Templeton et al., 2006).

In contrast to methanotrophy, methanogenesis showed much more temperature dependence, with Q₁₀ values varying from 5.3 to 16 between 10 and 25°C (Dunfield et al., 1993). Yvon-Durocher et al. (2014) using meta-analyses reported the temperature dependencies of methanogenesis in pure culture of methanogens and anaerobic microbial communities as well as a wide range of ecosystems (aquatic, wetland and rice-paddy ecosystems). Due to the markedly positive correlations between CH₄ production with temperature, it was suggested that global warming may have a greater effect on CH₄ emissions from various ecosystems (Yvon-Durocher et al., 2014).

1.3.3 Soil pH

Methanogens exhibit a more sensitive response to soil pH than methanotrophs, which can be tolerant over a wide range of pH values (Dunfield et al, 1993; Shukla et al., 2013). Methanogens are more active in neutral (pH= 6.5–7.5) or slightly alkaline soil (Malyan et al., 2016), while several methanotrophs in pure culture can grow within the pH range from 5.0 to 9.0 (Amaral et al., 1995). This phenomenon was not only reported from pure culture incubation, studies also found that soil pH is not a very crucial factor controlling CH₄ oxidation (Kolb, 2009; Shukla et al., 2013; Xu et al., 2014), as consumption of atmospheric CH₄ in soils can be observed in a wide range of pH values (pH=3.5-8.0) (Kolb, 2009). In forest soils, pH is mostly below 6.0 whilst atmospheric CH₄ consumption is often high (Tate, 2015). It has been reported that the optimum pH of CH₄ oxidation varied from 4.0 to 7.5 in boreal forest soils (Saari, Rinnan and Martikainen, 2004), while the maximum CH₄ production rates were at

neutral pH conditions (Malyan et al., 2016). It was found soil acidity was significantly greater under coniferous tree stands than in deciduous tree stands in temperate soils (Rothe et al., 2002) and the net soil CH₄ uptake rate is usually negatively related to increasing alkalinity and acidity beyond the optimal pH range (Xu and Inubushi, 2009). The difference of soil CH₄ uptake between coniferous forests and deciduous forests might be explained by CH₄ and O₂ diffusion rates through organic horizons, soil acidification and concentrations of inhibitory compounds (Degelmann et al., 2009). However, researchers found that there were no differences of CH₄ diffusive flux through the litter layer and pH was similar in both European beech and Norway spruce forest soils, combined with the negligible ethylene accumulation in all soil types, suggesting the communities of methanotrophs may be the driver of the differences in soil CH₄ oxidation rates (Degelmann et al., 2009).. Jang et al. (2006) reviewed the results of 28 studies on CH₄ oxidation in various forest soils and concluded that pH is only a minor controlling factor in natural soils.

1.3.4 Soil nitrogen

Over the past 200 years, atmospheric N deposition has increased more than ten times than in pre-industrial times in temperate forests (Magnani et al., 2007; Janssens et al., 2010; Geng et al., 2017) and thus it is important to understand the effect of N addition on forest soil CH₄ exchange. A meta-analysis of forest ecosystem showed that N enrichment decreased CH₄-C uptake and had no effect on CH₄-C emission (Liu and Greaver, 2009), but the wealth of reports on inorganic N affecting forest soils CH₄ uptake both *in situ* and *in vitro* are not consistent. Some studies have shown that CH₄ oxidation rates decreased under additional N deposition in forest soils (Steudler et al., 1989; Butterbach-Bahl et al., 2002; Gundersen et al., 2012; Yang, Wang and Xu, 2017). Krause, Niklaus and Schleppei (2013) even found CH₄ shifted from a net sink to a net source under chronic low-dose N addition in a temperate forest, but the reason still remains unclear. It was suggested that N addition primarily affects the methanotrophic community in the soil by increasing osmotic pressure (King and Schnell, 1998; Bodelier and Laanbroek, 2004; Yang, Wang and Xu, 2017). The contrasting effect of increasing inorganic nitrogen on lignin decomposition and protein catabolism suggested that further studies on the impact of N addition on organic matter decomposition are needed (Lucas and Casper, 2008), though it was expected that inorganic N addition may not promote higher methanogenesis, as methanogens access to smaller organic compounds would be inhibited

by the decrease in organic matter decomposition (Aronson and Helliker, 2010). A forest site with lower atmospheric N deposition ($5\text{--}6 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), soil CH_4 oxidation rates were 1.4 times higher than in the forest site with higher atmospheric N deposition ($30 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) (Butterbach-Bahl et al., 1998). Meta-analysis showed the effect of N availability on CH_4 uptake in non-wetland soil depends on the concentration of N addition, with promoting effects in N (including organic and inorganic) input below $100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Aronson and Helliker, 2010). Similar results showed that CH_4 uptake was stimulated by low rate of urea fertilizer addition ($10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), while a high rate of N addition ($140 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) inhibited soil CH_4 uptake fluxes in a temperate forest in China (Geng et al., 2017). These results confirm that Bradford et al. (2001) found no significant N effect on net CH_4 oxidation in a temperate forest in the UK, which was explained by the low level of local elevated N deposition. Lab experiments showed the effect of N addition on forest soil CH_4 uptake may depend on CH_4 concentrations, with a promotion under low atmospheric CH_4 concentrations ($1.7\text{--}2.0 \text{ ppmv CH}_4$) and an inhibition under high CH_4 concentrations (300 ppmv CH_4), which was due to the different activity of methanotrophs at various CH_4 concentrations (Jang et al., 2011).

Researchers have also studied the effect of different N species, i.e. ammonium (NH_4^+) and nitrate (NO_3^-), on soil CH_4 exchange (Jang et al., 2011; Yang, Wang and Xu, 2017), but the effect of inorganic N on CH_4 emissions between forest soils and the atmosphere has not been fully understood to date. Several studies have shown that NO_3^- addition to forest soils exhibited a stronger inhibitory effect on CH_4 oxidation than NH_4^+ (Wang and Ineson, 2003; Reay and Nedwell, 2004; Mochizuki, Koba and Yoh, 2012), while Yang, Wang and Xu (2017) found that NH_4^+ , rather than NO_3^- , was the major factor contributing to the inhibitory effect of N input on CH_4 uptake in a temperate forest during a five-year *in situ* study in China. It was suggested that NO_3^- as an oxidant for denitrifiers that cannot only outcompete methanogens for substrate, which inhibits CH_4 production (Bodelier and Steenbergh, 2014), but also added NO_3^- and nitrite (NO_2^-) produced by nitrification or denitrification processes are probably toxic to methanotrophs (Schnell and King, 1994; Wang and Ineson, 2003). While NH_4^+ can inhibit CH_4 oxidation by competing for methane mono-oxygenase (MMO) (Bédard and Knowles, 1989) and hydroxylamine and nitrite that are released during methanotrophic ammonia oxidation can be toxic to the methanotrophs (Schnell and King, 1994). Meta-analysis indicated that different N form addition in soils could have similar results, as the form of N that results

may be different from actual N species added, due to various microorganisms that are capable of N transformation (Aronson and Helliker, 2010). What also increases the difficulty of studying the effect of N addition on CH₄ exchange between forest soils and the atmosphere are the discrepancies between the laboratory incubation (*in vitro*) and *in situ* CH₄ flux observations. As *in vitro* studies often showed immediate short-term (less than 21 days) N effects in upland as well as lowland soils, *in situ* studies mostly long-term (more than a year) N effects or no effect were observed (Bodelier and Laanbroek, 2004; Yang, Wang and Xu, 2017). However, the effect of N addition on soil-atmosphere CH₄ exchange in forests needs to investigate the underlying mechanisms and has to be assessed on a case-by-case basis.

1.3.5 Soil organic matter

Methanotrophs are classified as the sub-group of methylotrophic bacteria, which are able to use CH₄ and other C₁ compounds as their sole energy and C source (Trotsenko and Murrell, 2008; Dedysh and Dunfield, 2011). Studies have reported that some genus of methanotrophs are actually facultative and can also use organic carbon sources other than CH₄, such as methanol, formaldehyde, formate, acetate, succinate, pyruvate, malate, methyl halides or ethanol (Dedysh and Dunfield, 2011; Sullivan et al., 2013; Fender et al., 2012; Subke et al., 2018). It was suggested that there will be an increase in the amount of labile carbon in the future due to the continuing high N addition into forest soils (Fender et al., 2012). Schnell and King (1995) studied the effect of nine different kinds of C compounds on the incubated forest soil CH₄ oxidation, but no correlations were found. However, it was indicated that the addition of glucose reduced the net CH₄ uptake of the temperate forest soils (Fender et al., 2012; Wu et al., 2016). It was suggested that adding labile C sources can stimulate heterotrophic microbial processes and change the preferred utilization of organic compounds in methanotrophs, thus decreasing oxygen concentration and inhibiting CH₄ oxidation (Wieczorek, Drake and Kolb, 2011; Fender et al., 2012). Additionally, glucose can also be easily used by methanogens to produce CH₄ and the addition of glucose can change the microbial community toward more fungal than bacteria, which could decrease CH₄ uptake rate (Wu et al., 2016). It shows that soil organic carbon (SOC) can affect methane oxidation by influencing the activity of methanotrophs, but the studies of the relationship between CH₄ oxidation rates or methanotrophic population and SOC are not consistent (Shukla et al., 2013). Recent research has shown that high net soil CH₄ uptake was correlated to lower quality soil dissolved

organic matter (DOM) pools in a temperate forest watershed, but the relationship between CH₄ uptake and soil DOM remains unclear (Warner et al., 2018).

1.3.6 Tree species

The effect of tree species on soil CH₄ uptake in different forest types is difficult to understand, as tree species-related factors are typically associated with litter quality, morphology of organic horizons and root systems. Furthermore, tree species are considered to affect soil physical (e.g. moisture and temperature), chemical (e.g. organic matter content and pH) and biological properties (e.g. microbial communities and mycorrhizal fungal community) (Borken and Beese, 2006; Menyailo, Abraham and Conrad, 2010; Fender et al., 2013; Prescott and Vesterdal, 2013). By excluding potential abiotic factors such as litter fall, soil moisture, soil bulk density and etc., the results showed that root-induced effects of different tree species can significantly influence CH₄ uptake in temperate mixed forests, which could be explained by the impact of root exudates amount on CH₄ uptake and less concentration of NH₄⁺ in the planted rhizotrons compared to bare soil (Fender et al., 2013). Reay et al. (2005) indicated that both high and low affinity CH₄ oxidation capacities were greatly reduced in soils under alder than those under oak, Norway spruce and Scots pine in a temperate forest in the UK. The reason behind this reduction in CH₄ oxidation by the presence of alder was probably due to its unique N-fixing root nodules, but it is still unclear whether it is caused by the elevated soil NO₃⁻ contents or transient elevated NH₄⁺ or nitrite concentration.

It has been reported that CH₄ consumption rates are higher in deciduous soils than in coniferous species soils in boreal and temperate forests (Butterbach-Bahl and Papen, 2002; Borken, Xu and Beese, 2003; Menyailo and Hungate, 2003; Borken and Beese, 2006; Degelmann, Borken and Kolb, 2009; Barrena et al., 2013). The literature summarized that in temperate deciduous forests CH₄ consumption rates range between 0.82 and 12.32 kg CH₄-C ha⁻¹ y⁻¹, while in coniferous forests consumption rates vary from 0.66 up to 4.80 kg CH₄-C ha⁻¹ y⁻¹ (Butterbach-Bahl and Papen, 2002; Dalal and Allen, 2008; Dalal et al., 2008; Jang et al., 2006; Saggar et al., 2008; Skiba et al., 2009; Barrena et al., 2013). Although coniferous trees tend to inhabit in cooler climates than deciduous trees, researchers found under the same range of annual temperature in two study sites, soil CH₄ oxidation rates in mature beech forests were 2 and 5.5 times higher than in mature pine and Douglas fir forests, respectively (Barrena et al., 2013). This phenomenon may be attributed to higher nutrient turnover rates,

microbiology activity in deciduous species soils (Ambus and Zechmeister-Boltenstern, 2007) as well as the difference in input of litter, the organic layer structure, bulk density of the mineral soil and C and N sequestration rates within forest floor and mineral soil (Butterbach-Bahl and Papen, 2002; Hagen-Thorn et al., 2004; Vesterdal et al., 2008). Additionally, it was suggested that the higher concentration of chemical components such as monoterpenes, produced by coniferous species (e.g. spruce) may explain the lower rate of CH₄ oxidation (Maurer et al., 2008) and lower pH in the upper mineral soils of coniferous forest sites might potentially inhibit the activity or the population of methanotrophs (Borken et al., 2003). Degelmann et al. (2010) showed that the diversity and abundance of methanotrophs in spruce soils are lower than those of beech soils in three European temperate forests, which suggested methanotrophic activity is higher in deciduous forest soils. However, the results showed that the rates of atmospheric CH₄ consumption were greatly affected by tree species, although the composition of high-affinity methanotrophs was not influenced by Siberian tree species (Menyailo et al., 2010). In contrast to other studies, Liu et al. (2014) found no significant differences in net CH₄ uptake over two years among any different tree species in natural temperate regenerated forests in China, which is in agreement with the study in afforested soils in Denmark (Christiansen and Gundersen, 2011). These two cases might be explained by the difference in soil types that have a history of human disturbance, e.g. cultivation, in which soils showed increasing N availability and decreasing bulk density after afforestation (Christiansen and Gundersen, 2011).

1.3.7 Mycorrhizae

Ectomycorrhizal (ECM) fungi are a group of microorganisms that can not only link plant roots to the surrounding soil environment, but also support a diverse microbial community in the rhizosphere of plant roots via their extensive extraradical mycelia (Fransson et al., 2016). As the significant role of ECM fungi in the circulation of autotrophic C and nutrients in forest soils has become clear (Subke et al., 2011; Heinemeyer et al., 2012), there is a need to understand the response of CH₄ oxidation rates to autotrophic C supply belowground. It was reported that WFPS is the major driver of CH₄ uptake in temperate forest soils, other than mycorrhizal associations (Meier et al., 2016), but the opposite results were shown by Subke et al. (2018). In the presence of ECM hyphae, net CH₄ uptake in temperate forest soils during summer was about 40% higher than in the bulk soil. It was suggested that soil moisture was unlikely to be

the main cause for this pattern, as the variations in soil moisture content were much less than the variations in CH₄ flux among the treatments. It was hypothesized that this phenomenon may be attributed to the differences in methanotrophic populations among treatments, because methanotrophs can use alternate organic labile C (e.g. methanol, formaldehyde, formate) and/or more nutrients produced by ECM fungi for methanotroph growth (Hanson and Hanson, 1996; Fransson et al., 2016; Subke et al., 2018). However, Burke et al. (2012) found a negative correlation between methanotrophs and fungal biomass and enzyme activity in forest soils, suggesting soil fungal biomass and fungal activity can influence the distribution of the methanotrophic group. The relationship between soil fungal biomass and the structure and distribution of methanotrophs which can influence soil CH₄ uptake needs further understanding.

1.3.8 Biochar

In order to enhance the removal of greenhouse gases from the atmosphere, the addition of biochar to soils was considered an effective climate change mitigation strategy (Fawzy et al., 2020). Biochar is a porous, charcoal-like, material generated from the thermal conversion of organic biomass (feedstock) under low oxygen pyrolysis conditions (Abbott et al., 2018). Compared to agroecosystems, few studies have focused on the biochar effect on soil CH₄ uptake in forest ecosystems (Li et al., 2018). Biochar application into forest soils can potentially alter soil physical (e.g., soil bulk density, soil porosity and soil water holding capacity), chemical (e.g., soil pH, soil organic carbon pools and soil nutrient availability) and microbial properties (e.g., microbial biomass and microbial community structure) (Li et al., 2018). It was found that the application of biochar into forest soils significantly increased soil CH₄ uptake, which may be due to the enhanced soil CH₄ oxidation rates (Yu et al., 2013). Biochar amended soils are more beneficial for methanotrophs by providing lower soil bulk density, higher soil porosity and better soil aeration, which leads to greater substrate availability and the increase in the soil CH₄ oxidation activity (Yu et al., 2013; Brassard, Godbout and Raghavan, 2016; Li et al., 2018). However, current studies showed that the effect of biochar addition on CH₄ uptake in temperate forest soils was not consistent, which presented no effect (Malghani, Gleixner and Trumbore, 2013; Sackett et al., 2015) and negative effect on soil CH₄ uptake (Hawthorne et al., 2017; Cui et al., 2021). It was observed that temperate forest soils with biochar addition have switched from a net CH₄ uptake to a CH₄ source after

a 49-day incubation (Cui et al., 2021). The decrease in soil CH₄ uptake after biochar amendment could be explained by the competition of organic compounds with atmospheric CH₄ as substrates for methanotrophs (Cui et al., 2021; Ji et al., 2018). More studies are needed to identify the underlying mechanism of biochar effect on forest soil CH₄ uptake.

1.3.9 Diurnal and seasonal variation

The diurnal pattern of CH₄ flux in forest soils is various. Studies showed that there was no clear diurnal CH₄ fluctuation in forested organic boreal soils, although the site was a net CH₄ sink (Maljanen et al., 2001). But it was found that CH₄ flux showed a diurnal variation in a temperate forest site, with a higher night-time CH₄ uptake rate and lower daytime CH₄ uptake rate (Dong et al., 2003). Similar results were also reported by Subke et al. (2018). It was hypothesized that diurnal changes in CH₄ uptake in temperate forest soils were associated with diurnal shift in atmospheric CH₄ concentration. But further studies need to identify the actual drivers of CH₄ uptake, as confounding covariance of air temperature and atmospheric CH₄ concentration may obscure the correlations.

Researchers have shown that *in situ* measurements of CH₄ uptake in temperate forest soils usually follows a clear seasonal pattern, with the highest rates during summer or the growing season (May-October) and the lowest rates during winter or the dormant season (November-April) (Butterbach-Bahl and Papen, 2002; Borken and Beese, 2006; Borken et al., 2006). It has shown that CH₄ uptake was 31-37% higher during the growing season than winter among all stands of a temperate forest in Germany (Borken and Beese, 2006). It was indicated that CH₄ seasonal variation is mainly related to soil water contents and soil temperature, but these drivers are often interacting which makes it difficult to separate their direct effect on CH₄ uptake (Butterbach-Bahl et al., 2002; Borken and Beese, 2006; Inclán et al., 2012). It was reported that about 80% of the seasonal variations in CH₄ uptake in a temperate soil can be explained by soil temperature and soil water content by multiple linear regressions, which showed that CH₄ uptake rate was stimulated by higher soil temperature and drier soil conditions (Ueyama et al., 2015). Higher soil moisture in spring compared to summer may explain lower CH₄ uptake rates during spring than in summer in temperate forest soils in the UK (Yamulki and Morison, 2017). However, the study of fifteen humid temperate forests in Japan indicated that seasonal variation of CH₄ uptake rate was mainly correlated with soil temperature other than soil water contents, which can be explained by the specific soil

properties with high porosity and aeration of topsoil (Ishizuka et al., 2009). In addition, studies have shown that seasonal fluctuation of CH₄ fluxes can also be driven by soil diffusion, as CH₄ uptake activity can be inhibited during frost periods and snow cover in winter (Guckland, Flessa and Prenzel, 2009). It was reported that CH₄ uptake rate was lowest under a snow cover during December to March, whereas CH₄ uptake rate was highest during summer drought in temperate forest soils (Borken and Beese, 2006). However, studies have shown that there was no significant relationship between seasonal variation and CH₄ fluxes over two years of measurement in temperate forest soils, although all sites acted as net CH₄ sinks and the reasons behind this phenomenon were still unclear (Jang et al., 2011; Liu et al., 2014).

1.4 Methane exchange with living trees

Although forests soils have been widely considered as one of the major sinks of CH₄ in the global budget, recent work indicates that trees have the potential to exchange CH₄ in upland forests (the term 'upland forests' in this thesis refers to the forests on well-drained, mineral soils, which usually is a net soil CH₄ sink). Since Keppler et al. (2006) have published the first debated observations that CH₄ might be produced abiotically in aerobic living intact C₃ and C₄ plants and leaf litter in forests, which is regulated by sunlight, temperature and physiological activity. Although it was indicated that structural plant component pectin may explain the *in situ* formation of CH₄ in plants, more details and investigations need to proceed (Keppler et al., 2006). Following researchers have widely discussed the possibility of the underlying mechanism of this process and CH₄ emission rates from plant tissue. Later reviews concluded that the phenomenon of aerobic CH₄ production in plants does occur (Keppler et al., 2009; Bruhn et al., 2012), but pectin may not be the only contributing precursor, as stimulating factors such as increasing ultraviolet (UV) radiation, increasing temperature, increased cutting injuries and secondary impact of the increase in the reactive oxygen species production under various types of stress (e.g. heat, UV radiation and wounding/cutting) may cause the difference in plant CH₄ release (Bruhn et al., 2012). Although the current knowledge for upscaling aerobic CH₄ into a global budget is still inadequate, studies indicated that the global aerobic CH₄ emissions by terrestrial vegetation based on leaf- and plant-based estimates may only account for a quarter of that estimated by Keppler et al. (2006) (62-236 Tg year⁻¹) (Kirschbaum et al., 2006; Parsons et al., 2006; Butenhoff and Khalil, 2007).

Some reports have shown that the living stems and shoots of trees can also be substantial sources of CH₄ fluxes in both boreal and temperate upland forests (Covey et al., 2012; Wang et al., 2016; Machacova et al., 2016; Warner et al., 2017; Pitz and Megonigal, 2017; Maier et al., 2018; Pitz et al., 2018; Barba, Poyatos and Vargas, 2019; Moldaschl et al., 2021; Barba et al., 2021). Recent research showed *in situ* tree stem mean CH₄ fluxes in temperate upland forests ranging from 6.3 ± 12 to $190 \pm 34 \mu\text{g m}^{-2} \text{ stem h}^{-1}$, which is a similar range to those observed in wetland forests. It was suggested that tree stem CH₄ emissions from forests can offset CH₄ consumption by soils and may switch the forest from a net CH₄ sink to a net source (Pitz and Megonigal, 2017; Pitz et al., 2018). However, the degree to which stem emissions from temperate forests can offset the soil sink is still highly uncertain, as different studies showed a wide range of estimates within varied ecosystems and tree species, which highlights a need for a better understanding of this process from local to global scales and re-evaluate the global budget of CH₄ sources and sinks (Wang et al., 2016; Warner et al., 2017; Pitz and Megonigal, 2017).

1.4.1 Underlying mechanisms of tree stem CH₄ emissions

Basically, there are mainly two assumptions about the mechanisms of CH₄ emissions from living tree stems, which depend on the forest ecosystem (wetland or upland forest soils) and tree species (containing dry and dense or wet and porous wood) (Yip et al., 2018). It is assumed that the source of tree-emitted CH₄ in floodplain and wetland forests is due to biological production in anoxic saturated soils or dissolved in groundwater which is subsequently absorbed by roots and then transported in stems through intercellular spaces and aerenchyma tissue or via the transpiration stream, with tree stems and leaves acting as major conduits for CH₄ emissions (Terazawa et al., 2007, 2015; Rice et al., 2010; Gauci et al., 2010; Pangala et al., 2013, 2015).

However, it was reported that CH₄ emissions from living tree stems in temperate upland forests are produced biologically *in situ* inside the trees themselves (Covey et al., 2012; Wang et al., 2016). Bark, sapwood, and heartwood are three layers of radial wood of tree trunks. Wang et al. (2016) found a higher CH₄ concentration in the heartwood of *Populus davidiana* than other wood layers, regardless of whether the heartwood is rotten or not, indicating significant barriers which inhibit radial diffusion of CH₄ from the heartwood to the atmosphere. Recent studies supported the concept of living trees themselves acting as a CH₄

source (Yip et al., 2018; Li et al., 2020; Barba et al., 2021). Yip et al. (2018) found that wood environments of *Populus deltoides* had a mean 34% relative abundance of methanogens in heartwood and 13% in sapwood environments. It was indicated that the presence of methanogenic archaea (*Methanobacterium*) explained the high concentration of CH₄ production in the heartwood of *Populus canadensis* (Li et al., 2020). The observation of high CH₄ concentrations inside the heartwood of *Carya cordiformis* and low CH₄ concentration from the soil profile in temperate upland forests, and the evidence of CH₄ production in wood incubations suggested that the origin of tree stem CH₄ emissions is likely inside the trees themselves (Barba et al., 2021).

Based on the current studies, the underlying mechanism of CH₄ emissions in living tree stems is still unclear. Pitz and Megonigal (2017) suggested that CH₄ emissions in living upland trees may come from various sources rather than a single source, which includes microbial production inside the tree stem (Mukhin and Voronin, 2011; Covey et al., 2012; Wang et al., 2016), and in subsurface soils or groundwater (Megonigal & Guenther, 2008; Machacova et al., 2016; Maier et al., 2018) and non-microbial UV-driven production by leaves and other tree surfaces (Keppler et al., 2008).

1.4.2 Tree stem CH₄ uptake

Although most studies show net CH₄ release from tree stems (Covey et al., 2012; Machacova et al., 2016; Wang et al., 2016, 2017; Pitz and Megonigal, 2017; Warner et al., 2017; Pitz et al., 2018), Sundqvist et al. (2012) observed *in situ* net uptake of CH₄ by branches of four different trees species (*Picea abies*, *Betula pubescens*, *Sorbus aucuparia* and *Pinus sylvestris*) in a boreal forest using an automated branch chamber. Phyllosphere microorganisms located on the leaves might explain the CH₄ consumption. In addition, laboratory measurements indicated that the CH₄ sink was likely located in the leaves, which showed a positive relationship between leaf CH₄ uptake rate with photosynthetically active radiation and stomatal conductance (Sundqvist et al., 2012; Covey and Megonigal, 2019). It was estimated that CH₄ uptake by the canopy of forests may play an equally significant role as CH₄ uptake by soils on a global scale (Sundqvist et al., 2012), but limited observations of leaf and stem CH₄ consumption were reported in upland temperate forests. Welch, Gauci and Sayer (2018) found tree stem CH₄ uptake in tropical forest on mineral soils, which may probably be due to the active consumption by epiphytic or endophytic bacteria (Raghoebarsing et al., 2005; Van

Aken et al., 2004). The gradient measurements of CH₄ exchange in a boreal forest displayed a diurnal pattern with lower net emissions at the daytime, correlating well with gross primary production, which suggested CH₄ uptake was from the canopy. However, it was not possible to distinguish CH₄ uptake by soil from uptake by vegetation, as the gradient measurements of gas flux were positioned above the canopy (Sundqvist et al., 2015). Recent studies provided further evidence of CH₄ uptake inside tree stems and shoots. Results have shown that novel CH₄ oxidising monooxygenases were detected from Norway spruce (*Picea abies*) shoot in boreal upland forests (Putkinen et al., 2021), and methane oxidising bacteria were observed inside the heartwood and sapwood of poplar (*Populus* sp.) in a subtropical upland forest (Feng et al., 2022) and from bark of *Melaleuca quinquenervia* in subtropical lowland forests (Jeffrey et al., 2021a). In addition, it was indicated that methane oxidising bacteria within lowland tree stems exhibited a novel CH₄ sink, which oxidised around one-third of stem CH₄ flux derived from soil (Jeffrey et al., 2021b). The intriguing mechanism of stem and shoots CH₄ uptake requires further investigation.

1.5 Factors of CH₄ exchange in living trees

1.5.1 Tree species and tree size

Studies have shown that stem CH₄ emissions differ among tree species in temperate forests (Covey et al., 2012; Wang et al., 2016, 2017; Warner et al., 2017; Pitz et al., 2018). Two possible reasons have been suggested for this. One reason is species-specific differences in disease resistance, for example, susceptibility to fungal-mediated heart rot. The decay of heartwood in living trees caused by the colonization of the fungal community and anaerobic decay may vary by tree species and within individuals (Covey et al., 2012; Warner et al., 2017). Another is wood anatomy, such as wood vessel structure, wood density, lenticel density, transpiration rates and sap flow rates (Pitz et al., 2018). Pangala et al (2014) found the effect of the wood-specific density and lenticel density on CH₄ fluxes in wetland trees. Furthermore, anatomical differences between gymnosperm and angiosperm species (e.g. tracheid vs vessels; softwood vs hardwood) may influence gas diffusion or microbial communities (Barba et al., 2019). It was indicated that nonstructural carbohydrates (NSCs) which are free sugars and starches stored in wood (Dietze et al., 2014) are a C source of methanogens in living tree stems (Covey et al., 2016; Covey and Magonigal, 2019). Compared to gymnosperms, Hoch et al. (2003) observed higher NSC stem sapwood concentrations in angiosperms. Studies have

reported that gymnosperm species showed lower tree stem CH₄ emissions than angiosperm species in upland forests, although there were few published studies of gymnosperm species in boreal upland forest sites (Machacova et al. 2014, 2016; Table 1.2). However, Pitz and Megonigal (2017) found no significant relationship between stem CH₄ emissions and seven studied tree species in temperate forests. Tree stem CH₄ emissions from various tree species in upland forests are shown in Table 1.2, which indicates large variations in tree stem CH₄ flux within different tree species.

It is suggested tree stem diameters may have physical effects on gas diffusion and thus influence CH₄ production or transport (Barba et al., 2019; Covey and Megonigal, 2019). But the effects of tree stem diameters on stem CH₄ emissions vary in different forest ecosystems. It was shown that stem CH₄ fluxes had a positive relationship with tree stem diameter in upland temperate forest (Wang et al., 2017; Pitz et al., 2018; Barba et al., 2021), while other studies in wetland forests reported the opposite (Pangala et al., 2013, 2015). It was suggested that the effect of small and large trees on stem CH₄ emissions may be due to differences in root morphology and biomass, as bigger trees have larger and deeper root systems that may tap into anaerobic soils or groundwater (Pitz et al., 2018). In addition, when tree stem CH₄ source was biologically produced from heartwood, stem CH₄ emissions were positively related to the ratio of heartwood diameter to stem diameter (Wang et al., 2017). However, molecular analysis showed that tree diameter at breast height (DBH) was inversely correlated to methanogen abundance of *Populus deltoids* (Yip et al., 2018). Despite of tree diameter, further studies need to focus on tree ages within a species, as older trees may have more methanogens established in the heartwood (Barba et al., 2019) and are more likely related to heart rot and have larger standing-wood volumes, which may produce more CH₄ (Covey et al., 2012). However, young trees can also be affected by heart rot, especially in tropical forests (Covey et al., 2012). Pangala et al. (2015, 2017) found higher stem CH₄ emissions from young trees than from mature trees in wetland forests.

Table 1. 2 Tree stem CH₄ emissions from various tree species (Pitz et al., 2018)

Reference	Ecosystem Type	Forest type	Plant community	Tree species	CH ₄ flux (mean ± SD) (µg m ⁻² h ⁻¹)
Covey et al. (2012)	upland	Temperate	gymnosperm	<i>Pinus strobus</i> L.	190 ± 34 (Modeled from internal concentration)
				<i>Tsuga canadensis</i> L.	
			angiosperms	<i>Betula lenta</i> L.	
				<i>Acer rubrum</i> L.	
				<i>Quercus rubra</i> L.	
Wang et al. (2016)	Temperate	angiosperms	<i>Populus davidiana</i>	85.3 (upper plot) 103.1 (lower plot)	
			<i>Carya cathayensis</i>	NA	
			<i>Larix gmelinii</i>	NA	
Machacova et al. (2016)		Boreal	gymnosperm	<i>Pinus sylvestris</i> L.	0.005 ^{med}
Warner et al. (2017)		Temperate	angiosperms	<i>Fagus grandifolia</i>	6.3 ± 12
				<i>Liriodendron tulipifera</i>	
				<i>Nyssa sylvatica</i>	
				<i>A. rubrum</i>	
				<i>Betula lenta</i>	
				<i>Quercus spp.</i>	
Pitz and Megonigal. (2017)		Temperate	angiosperms	<i>Fagus grandifolia</i>	25.44 ± 14.08
				<i>Liriodendron tulipifera</i>	
				<i>Liquidambar styraciflua</i> L.	
				<i>Q. velutina</i> Lam.	
				<i>Acer rubrum</i> L.	
				<i>C. tomentosa</i> (Lam.) Nutt.	
				<i>Q. michauxii</i> Nutt.	
				<i>Fagus grandifolia</i>	
Pitz et al. (2018)		Temperate	angiosperms	<i>Fagus grandifolia</i>	68.8 ± 53.6

Barba, Poyatos and Vargas (2019)	Temperate	angiosperms	<i>Liquidambar styraciflua</i>	16.13 (150 cm stem height)	
			<i>Liriodendron tulipifera</i>		
			<i>Quercus michauxii</i>		
			<i>Fraxinus pennsylvanica</i>		
			<i>Acer rubrum</i>		
			<i>Carpinus caroliniana</i>		
			<i>Carya tomentosa</i>		
			<i>Quercus velutina</i>		
			<i>Carya cordiformis</i>		26.50 (75 cm stem height)
			<i>(Wangenh.) K.Koch</i>		
Barba et al. (2021)	Temperate	angiosperms	<i>Carya cordiformis</i>	146.30 (automated measurements)	
				13.82 (manual measurements)	

NA, not available
Med, median

1.5.2 Soil moisture, water table depth and stem water content

It was reported that stem CH₄ fluxes of silver birch (*Betula pendula*) and Scots pine trees (*Pinus sylvestris*) at wet plots were higher than those at dry plots in boreal upland forests, showing a positive relationship with soil moisture (Machacova et al., 2014, 2016). Similar positive response of tree stem CH₄ emissions to soil moisture was also observed in temperate upland forest (Maier et al., 2018; Barba, Poyatos and Vargas, 2019; Barba et al., 2021; Moldaschl et al., 2021). This phenomenon may indicate the source of tree stem CH₄ at wetter area is from belowground, with a strong positive relationship between stem CH₄ fluxes with forest floor CH₄ fluxes and soil volume water content on wetter plot (Machacova et al., 2016). It was suggested areas with higher soil moisture can enhance soil CH₄ production in deeper soil layers, while inhibiting soil CH₄ oxidation in upper soil layers due to the reduction of soil diffusivity. In addition, higher soil moisture can increase stem respiration and transpiration stream, leading to higher stem CH₄ emissions (Machacova et al., 2016; Barba, Poyatos and Vargas, 2019). However, other reports found no influence of soil moisture on stem CH₄ emissions in temperate (Pitz and Megonigal, 2017; Warner et al. 2017; Pitz et al., 2018) and tropical forests (Welch, Gauci and Sayer, 2018).

Researchers found that tree stem CH₄ emission rates were related to water-table level from *Alnus glutinosa* saplings grown under two artificially controlled water-table positions, with significant stem CH₄ emissions under high water-table level mesocosms and negligible stem CH₄ emissions under low water-table level mesocosms (Pangala et al., 2014), which suggested the CH₄ source is from the saturated groundwater and transporting CH₄ via transpiration stream or diffusion to the atmosphere. Similar results were also reported that stem CH₄ emissions were negatively related to the depth to water table in temperate forests (Terazawa et al., 2015; Pitz et al., 2018). It was indicated that plant rooting depth can control the magnitude of plant-mediated CH₄ emissions under varying water-table conditions, with less substrates supplied by shorter and fewer roots into anaerobic CH₄ production zone and more substrates into the aerobic CH₄ oxidation zone, reducing CH₄ production rate (Waddington, Roulet and Swanson, 1996; Pangala et al., 2015). The rooting depth and structure of various tree species in different forest ecosystems need to be further investigated.

Compared with wetter soil areas in forests, CH₄ emitted from trees at drier areas may be generated from anaerobic production inside the wood (Mukhin and Voronin, 2009; Mukhin

and Voronin, 2011; Covey et al., 2012; Wang et al., 2016). Wang et al. (2017) found that stem CH₄ emissions in living trees in upland forests were controlled by water content in the heartwood and CH₄ was effectively produced when water content was above a threshold of 45-53% w/w, which created anoxic conditions and favoured methanogenesis. However, it was found that wood moisture content was not significantly correlated to the relative abundance of known methanogenic taxa of *Populus deltoides* (Yip et al., 2018). It was suggested that other factors may play a vital role in stem CH₄ emissions than wood water content when it is above a certain moisture threshold within wetwood trees such as *Populus deltoides* (Yip et al., 2018).

1.5.3 Stem height

Most studies have shown that tree stem CH₄ emissions in both wetland and upland forests declined with increasing stem height varied from 10 to 465 cm above the ground, which may be explained by the assumption of tree stem CH₄ source from deep layers of anaerobic soils (Terazawa et al., 2007; Pangala et al., 2013, 2014, 2017; Wang et al., 2016; Pitz and Megonigal, 2017; Pitz et al., 2018; Barba, Poyatos and Vargas, 2019; Jeffrey et al., 2019; Jeffrey et al., 2021b). In contrast to other studies, one beech tree located in a temperate upland forest site was observed that stem CH₄ emissions increased with stem height, which was still unclear and lack of substantial evidence due to a limited number of observed trees (five representative trees per site) (Maier et al., 2018). A recent study reported that the opposite effect of tree height was observed on tree stem CH₄ emissions in two tree species (*Fraxinus excelsior* and *Populus alba*) in temperate upland forests (Moldaschl et al., 2021). Further studies need to focus on a larger number of trees at various stem heights of different tree species and forest ecosystem types to understand the underlying mechanism.

1.5.4 Sap flow

Studies have confirmed the correlations between sap flow and tree stem CO₂ flux (Teskey et al., 2008) (the term of 'stem CO₂ flux' in this thesis refers to the CO₂ flux from tree stems released to the atmosphere). It was suggested that measuring transpiration (i.e. sap flow rate) coupled with tree stem emissions could better understand the origin of stem CH₄ flux (Barba et al., 2019). Barba, Poyatos and Vargas (2019) reported that temporal stem CH₄ emissions were positively related to sap flow at diurnal and seasonal scales in temperate upland forests,

directly linking stem emissions with stem water transported from belowground via the transpiration stream and suggesting the origin of CH₄ is produced in soils. Similar results were shown by Machacova et al. (2016) with the observation of a positive relationship between stem CH₄ flux and sap flow in boreal forest, which also suggested the partial soil origin of pine-emitted CH₄, rather than the radial diffusivity of CH₄ within stems.

It is speculated that when the source of stem CH₄ is from heartwood, CH₄ can be partially dissolved into the sap and then emitted through the stem with radial diffusivity. The xylem would act as a barrier rather than a transport channel (Barba et al., 2019). Wang et al. (2017) believed that a large quantity of water discharged from the drilled holes of wet heartwood may not come from the sap flow, which showed the relatively small quantity of water, but rather may be related to the wet belowground environments. And it was found that in temperate upland forests when the pressure of water in the heartwood of *Populus davidiana* with substantial CH₄ was relieved, CH₄ in mini bubbles within water was immediately released into the atmosphere.

1.5.5 Wood density

The variations in stem CH₄ emissions of individual trees between species could be attributed to wood density, which reflects the wood porosity and anatomical composition, consequently affecting wood gas diffusivity (Pangala et al., 2013; Wang et al., 2016; Barba et al., 2018). Negative correlations between stem CH₄ emissions and heartwood density at both daily and seasonal scales were reported in a deciduous temperate upland forest, indicating wood density may influence pore space for CH₄ diffusion (Wang et al., 2017). Similar results of inverse relationships between tree stem CH₄ flux to wood density were observed in wetland trees (Pangala et al., 2013, 2015). Higher wood density can enhance stem anoxia by decreasing O₂ diffusion and thus favoring CH₄ production inside, but CH₄ diffusion to the stem surface can also be inhibited, which makes it difficult to explain the effect of wood density on stem CH₄ emissions (Covey and Megonigal, 2019). Not only wood density, but stem lenticel density can also affect stem and root aerenchyma tissues, which in turn may change the tree-mediated CH₄ emissions. Positive relationships between tree stem CH₄ flux rate and stem lenticel density were found from *Alnus glutinosa* saplings grown under lab-controlled conditions (Pangala et al., 2014).

1.5.6 Temperature

Studies about the effect of temperature on stem CH₄ fluxes are not consistent, as some reports showed a positive relationship between tree stem CH₄ emissions and temperature in temperate upland forests on both daily (Barba, Poyatos and Vargas, 2019) and seasonal scales (Wang et al., 2016; Barba, Poyatos and Vargas, 2019). While others showed no relationship in temperate upland forests (Pitz and Megonigal, 2017; Warner et al., 2017; Pitz et al., 2018) and the mechanism behind this phenomenon is still unclear.

1.5.7 Stem CO₂ flux

Researchers have suggested that measurements of stem CO₂ flux simultaneously with stem CH₄ flux can help to understand the pathway of stem CH₄ flux (Barba et al., 2019). Compared to stem CH₄ flux, the mechanism of stem CO₂ flux has been more widely studied (Teskey et al., 2008, 2017). There are two sources of stem CO₂ flux and the majority of stem CO₂ flux is originated from the respiring cells in the stem and roots (Teskey et al., 2008). In addition, stem CO₂ flux can also derive from the rhizosphere, which is originated from microbial or root respiration, dissolved in soil water and can be absorbed by roots and transported into the stem via sap flow or transpiration (Teskey et al., 2008). Results have shown that a positive correlation between stem CH₄ and CO₂ fluxes was observed over a growing season in a temperate upland forest (Barba et al., 2021). This correlation may be explained by gas diffusivity heterogeneity through the wood, which might similarly affect both gases by common physical barriers (Barba et al., 2019; Megonigal, Brewer and Knee, 2020). However, the large variations in tree stem CH₄ flux make it complicated to model tree stem CH₄ flux only based on stem CO₂ flux (Barba et al., 2021). Future studies are suggested to take measurements of both stem CH₄ and CO₂ fluxes as well as the wood tissue anatomy to identify the pathway of stem CH₄ flux.

1.5.8 Diurnal and seasonal variations

In upland temperate forests, it was observed that a single *Liriodendron tulipifera* tree showed a strong diurnal pattern in stem CH₄ flux with peak emissions in the late afternoon, using an automated system for high-frequency gas flux measurements, which suggested tree stem CH₄ emissions may from soils via transpiration (Pitz and Megonigal, 2017). Similar results were demonstrated by Barba, Poyatos and Vargas (2019), showing temporal diurnal variations

associated with sap flow and temperature in stem CH₄ fluxes of a single *Carya cordiformis* tree by an automated chamber-based high-frequency analyser, but this trend was not consistent during the growing season. In contrast, other studies reported no diurnal stem CH₄ variations in temperate upland, floodplain and riparian forests by using manual gas flux measurements (2-4 weeks frequency) (Wang et al., 2016; Terazawa et al., 2015; Schindler et al., 2021). High-frequency measurements of tree stem CH₄ flux are highly recommended for studying the diurnal pattern (Barba et al., 2019; Covey and Megonigal, 2019). It is speculated a systematic bias may be potentially introduced via manual measurements, which lack of capturing the high-variability of stem CH₄ flux as well as limited the frequency of stem flux observations (Barba et al., 2019; Barba, Poyatos and Vargas, 2019). In addition, *in situ* high-frequency measurements of diurnal variations can potentially reveal the pathway of tree stem CH₄ emissions, indicating CH₄ transporting from soils via transpiration, rather than from heartwood by diffusivity across the stem (Covey and Megonigal, 2019). However, it is difficult to differentiate these two pathways only by the diurnal pattern, as they may interact with each other before CH₄ emits from a tree surface (Pitz et al., 2018; Barba et al., 2019; Covey and Megonigal, 2019). Pangala et al. (2014) did not observe a diurnal pattern in stem CH₄ emissions from *Alnus glutinosa* saplings using a high-frequency analyser, indicating passive diffusion was the dominant pathway for stem CH₄ transport.

Various drivers are currently found that can contribute to seasonal variations in tree stem CH₄ emissions based on forest ecosystem and environmental conditions. In a temperate upland forest, it was reported that a single *Carya cordiformis* tree showed similar stem seasonal CH₄ fluxes at both upper and lower stem heights, which can be explained by temperature, sap flow and soil water content (Barba, Poyatos and Vargas, 2019). Wang et al. (2017) found a negative relationship between stem CH₄ emissions and heartwood density at a seasonal scale in upland forests, which may partly be due to temperature that can affect CH₄ production from methanogenesis in heartwood. While in the wetter forests such as a temperate floodplain forest and a temperate wetland forest, the drivers of differences in seasonal variations in stem CH₄ flux among individual trees of similar tree species might be explained by the different variations of water table depth nearby (Terazawa et al., 2015) or soil temperature and pore-water CH₄ concentration (Pangala et al., 2015). However, a small or null effect of seasonal patterns on stem CH₄ emissions was also found in floodplain, wetland

and upland forests by manual measurements (Terazawa et al., 2007; Warner et al., 2017; Pitz and Magonigal, 2017; Pitz et al., 2018; Welch, Gauci and Sayer, 2018; Moldaschl et al., 2021). It was suggested that compared to manual measurements (manual chamber, monthly frequency), high temporal frequency measurements (automated chamber, hourly frequency) are better at capturing the large temporal variability of tree stem CH₄ flux (Barba et al., 2019, 2021). Results have shown that combining manual and automated flux measurements of 18 hickory trees (*Carya cordiformis*) in a temperate upland forest, only automated measurements exhibited a seasonal trend in tree stem CH₄ flux which peaked at the end of summer and started decreasing around the end of autumn (Barba et al., 2021). Further studies are recommended to use high-frequency measurements of tree stem CH₄ flux to address the temporal variability.

1.6 Thesis aim, objectives and structure

This thesis focused on CH₄ and CO₂ fluxes from both soils and tree stems in temperate forests on mineral soils in the UK, and investigated the potential biotic and abiotic drivers, and tried to identify the underlying mechanism of tree stem CH₄ exchange. In terms of CO₂ as another key greenhouse gas, the measurements of CO₂ fluxes from soil and stems were also taken in this thesis to help with identifying drivers of stem CH₄ fluxes. Based on the knowledge gaps in the current literature on soil and stem CH₄ exchange in temperate upland forests, there were two broad aims of this thesis:

1. Study the effects of ectomycorrhizal mycelium and biochar on soil net CH₄ uptake and soil respiration, and their potential underlying abiotic drivers.

In chapter 2, a long-term (2012 to 2021) manipulation experiment in a temperate coniferous forest soil was carried out, in order to assess the effects of ECM mycelium presence and biochar application on soil net CH₄ uptake and soil respiration during the short-term (1-3 years) and long-term (3-9.5 years). We also studied the seasonal variations in soil CH₄ uptake and soil respiration and their potential underlying drivers.

2. Study tree stem CH₄ and CO₂ fluxes variations between tree species, at different stem heights, and quantify temporal variation to elucidate the underlying mechanism.

In order to unravel the underlying mechanism of tree stem CH₄ exchange in temperate upland forests, both field and lab experiments were performed. The high spatial and temporal variability of tree stem CH₄ flux was determined within different tree species and at different stem heights using both manual and high-frequency measurements. We also studied potential CH₄ production under anaerobic conditions and potential CH₄ oxidation rates under aerobic conditions of different trunk layers via wood incubation in the lab.

In chapter 3, rigid stem chambers were installed on 24 tree stems at two sites from a temperate, managed forest on mineral soil. We determined the variations of tree stem CH₄ and CO₂ fluxes within two species of contrasting anatomy, English oak (*Quercus robur*, deciduous broadleaf) and Japanese larch (*Larix kaempferi*, deciduous conifer) during a growing season by manual flux measurements.

In chapter 4, we used high-frequency measurements (automated chambers, 1.5 hourly frequency) to determine the high temporal variability of stem CH₄ and CO₂ fluxes from three white poplar (*Populus alba*) trees over a 2-month summer period in a temperate upland woodland, and explored the relationship between soil CH₄ uptake or production with stem CH₄ emissions by comparing high-frequency CH₄ (and CO₂) fluxes at different tree stem heights and from the soil.

In chapter 5, wood incubation experiments were carried out in the lab under both anaerobic and aerobic conditions, to determine the potential CH₄ production and oxidation rates in the three main layers of trunks (bark, sapwood and heartwood) and from areas with or without lenticels of white poplar (*Populus alba*) trees at different stem heights.

In the final chapter of the thesis, we presented a general discussion summarising the findings, focusing on opportunities for future research.

The hypotheses were:

1. The presence of ECM mycelium increases net soil CH₄ uptake and soil respiration during spring or earlier summer, but decreases soil CH₄ uptake during autumn and winter, because seasonal variations in temperature and nutrients change the ECM community structure.

2. Biochar application does not have significant long-term (more than 3 years) effects on net soil CH₄ uptake and soil respiration, but can decrease soil CH₄ uptake and CO₂ emission in the short-term (1-3 years) because it increases the soil nutrient availability.
3. Tree stem CH₄ and CO₂ fluxes are larger from English oak than those from Japanese larch due to different wood anatomy, while soil acts as a net CH₄ sink at both research sites.
4. Oak and larch tree stem and soil CH₄ and CO₂ fluxes exhibit seasonal variations, which are caused by environmental drivers (such as soil moisture, soil temperature and air temperature).
5. White poplar tree stem CH₄ and CO₂ fluxes both show a consistent diurnal pattern, with larger fluxes during the daytime than during the night-time.
6. White poplar tree stem CH₄ and CO₂ fluxes decrease with increasing stem height, because these fluxes are partially derived from the deeper layers of soil, whilst soils act as a net CH₄ sink.
7. All trunk layers of white poplar trees have the capacity to produce CH₄ under anaerobic conditions, and heartwood has the highest potential CH₄ production rate.
8. All trunk layers of white poplar trees have the capacity to oxidise CH₄ under aerobic conditions, and bark has the highest potential CH₄ oxidation rate.

2. The Effect of Ectomycorrhizal Mycelium and Biochar on Forest Soil Methane Uptake and Soil Respiration in a Temperate, Coniferous Forest

2.1 Abstract

The exchange of soil CH₄ and CO₂ fluxes in temperate forests plays a vital role in the global CH₄ and soil respiration budgets. The net soil CH₄ uptake and soil respiration can be driven by both abiotic and biotic factors. Ectomycorrhizal (ECM) fungi in forest soils act as a key role in biogeochemical cycling. Although biochar is considered a promising method for carbon capture, little is known about its interactions with the forest ecosystem. In this study, we measured soil CH₄ and CO₂ fluxes on 20 occasions during 2012 to 2021 in a temperate, coniferous forest in England. The experiments were established in 2011 with and without ectomycorrhizal fungi presence and biochar addition treatments. The presence of ECM mycelium exhibited inconsistent effects (positive, negative and no effect) on both soil CH₄ uptake and soil respiration during 2020 to 2021. However, analysis of the entire study period data showed the presence of ECM mycelium significantly decreased the cumulative CH₄ uptake over the long-term 8.5-9.5 years (Jul 2020-May 2021, P=0.041), but showed no effect on cumulative CO₂ flux in this study or from long-term data spanning 1-9.5 years. Biochar addition did not show any significant effect on soil CH₄ uptake and cumulative CH₄ uptake over 8.5 years of measurements, whilst tended to inhibit soil respiration only 4 times out of the 20 measurement dates over the entire study. Soil CH₄ uptake and soil respiration followed a similar seasonal trend over the years with the highest rates from June to November and lowest rates from December to May. Soil CH₄ uptake significantly increased with increasing soil temperature and decreasing soil moisture (P<0.001), while soil respiration only significantly increased with soil temperature (P<0.001).

2.2 Introduction

Methane (CH₄) is the second most important greenhouse gas (GHG) after carbon dioxide (CO₂), globally responsible for about 20-25% of the additional radiative forcing (RF) from 1750 to 2011 (Etminan et al., 2016). Soils are the only known terrestrial net CH₄ sink, accounting for approximately 4% of the global CH₄ sink (Saunio et al. 2020). This uptake of CH₄ is projected

to increase more than four times by 2100 ($82.7 \pm 4.4 \text{ Tg yr}^{-1}$) compared to 1900 ($17.1 \pm 2.4 \text{ Tg yr}^{-1}$), which was primarily driven by the increase in atmospheric CH_4 mole fraction (Murguía-Flores et al., 2021). Temperate forest soils are important biological CH_4 sinks. It was estimated that temperate forest soils contributed 27% to the total CH_4 uptake in global forest soils in 1981-2000 (Yu et al., 2017). In contrast to CH_4 flux, soil respiration - CO_2 release from the soil surface - is the second largest terrestrial carbon flux (IPCC, 2007), contributing to about 10% of the atmospheric CO_2 cycles annually (Reichstein and Beer, 2008). The mean soil respiration fluxes from the temperate humid evergreen and temperate humid deciduous on a global scale were 3.53 ± 0.15 and $2.77 \pm 0.17 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, respectively (Luysaert et al., 2007; Oertel et al., 2016).

Net CH_4 exchange between soils and the atmosphere largely depends on the net balance of two contrasting microbial processes – CH_4 production and CH_4 oxidation. Soils are considered net CH_4 sinks when the activities of methane oxidizers (methanotrophs) generally dominate over those of methane-producing archaea (methanogens) (Conrad, 2009). Soil respiration includes root, anaerobic and aerobic microbial respiration. Root respiration involves all the respiration processes in the rhizosphere, contributing ca. 50 % of the total soil respiration with the variation between 10 to 95% according to season and vegetation type (Hanson et al., 2000; Oertel et al., 2016). Net soil CH_4 uptake in temperate forests are driven by both abiotic (e.g. soil moisture, temperature, soil pH and soil nitrogen content) and biotic factors (e.g. tree species and mycorrhizae) (Tate, 2015; Malyan et al., 2016; Xu and Shang, 2016; Oertel et al., 2016). Compared to the effect of abiotic factors on soil CH_4 uptake and soil respiration, the effect of biotic mycorrhizae is still poorly understood.

2.2.1 Ectomycorrhizal fungi effect

In temperate ecosystems, ectomycorrhizal (ECM) fungi associated with tree roots can account for up to 80% of the fungal community and comprise one-third of the total microbial biomass in forests soils (Högberg and Högberg, 2002; Prescott and Grayston, 2013). ECM fungi can access organic and inorganic sources from the soils via the extramatricial mycelium (Smith and Read, 2008). Rather than acquiring the release of metabolic carbon, ECM fungi decompose soil organic matter (SOM) primarily for nitrogen mobilization in temperate forest ecosystems (Lindahl and Tunlid, 2015). This was supported by several field studies that ECM fungi can benefit from nitrogen in SOM (Averill & Hawkes, 2016; Cheeke et al., 2016), but it

was still unclear whether all ECM fungi have the ability to liberate nitrogen from SOM (Pellitier and Zak, 2017). Compared to nitrate, the uptake rate of ammonium by ECM fungi was almost 16 times higher in temperate forests, and ectomycorrhizal colonization rates can positively affect the uptake rates of ammonium and nitrate contents in soils (Liu et al., 2017), which may consequently have an impact on soil CH₄ uptake. It was reported that the addition of ammonium and nitrate can inhibit soil CH₄ oxidation in temperate forests soils (Wang and Ineson, 2003; Yang, Wang and Xu, 2017), but this depends on the concentrations of nitrogen applied in the soils. Based on a meta-analysis in non-wetland soils, the concentration limit of nitrogen addition (including organic and inorganic) was at around 100 kg N ha⁻¹ y⁻¹, above which soil CH₄ uptake tended to be inhibited (Aronson and Helliker, 2010). However, in the natural and unfertilized forest soils with low concentrations of nitrogen, the effect of the ammonification and nitrification rate on CH₄ oxidation in the rhizosphere was not observed (Meier et al., 2016).

ECM fungi can also benefit soil bacteria by providing nutrition (Nazir et al., 2010). Low molecular weight organic compounds such as organic acids and amino acids exuded from ECM fungi in the rhizosphere of Scots Pine (*Pinus sylvestris*) can support diverse microbial communities belowground (Fransson et al., 2016). Subke et al. (2018) found that the net CH₄ uptake and soil respiration in temperate forest soils during summer were higher with the presence of ECM hyphae. The results might be explained by providing more alternate organic labile carbon and/or nutrients by ECM fungi for methanotroph growth. However, soil samples collected in September from temperate hardwood forests indicated that methanotrophs were negatively correlated with all fungal biomass and enzyme activity, suggesting methanotrophs probably dominated in areas with low carbon and nutrient cycling rates (Burke et al., 2012). The contrasting finding results may be explained by the different sampling seasons. Further long-term (whole growing and non-growing seasons across years) studies are required to better understand the ECM fungi effect on soil CH₄ uptake and soil respiration, as ECM fungal community structure exhibited large temporal variation (Prescott and Grayston, 2013). The seasonal trend of ECM fungal community structure varied between individual morphotypes in temperate forests, where a number of morphotypes of ECM fungi were more abundant in winter than in summer, whilst the others exhibited the opposite pattern (Buée et al., 2005; Courty et al., 2008). The temporal changes in ECM fungi community could be

explained by root longevity, the different response to environmental variability (such as soil temperature, nutrients) and competition between ECM species for soil or tree resources (Courty et al., 2008; Burke et al., 2011).

2.2.2 Biochar effect

In order to enhance the removal of greenhouse gases from the atmosphere and increase soil carbon sequestration, the addition of biochar to soils was considered an effective climate change mitigation strategy (Fawzy et al., 2020). Biochar is a porous, charcoal-like, material generated from the thermal conversion of organic biomass (feedstock) under low oxygen pyrolysis conditions (Abbott et al., 2018). Compared to agroecosystems, few studies have focused on the biochar effect on soil CH₄ and CO₂ fluxes in forest ecosystems (Li et al., 2018). Cui et al. (2021) observed that temperate forest soils (coarse grains and a high quartz content) with spruce biochar addition have switched from a net CH₄ uptake to a CH₄ source after the short-term incubation (49 days). The decrease in soil CH₄ uptake after biochar amendment could be explained by the competition of organic compounds with atmospheric CH₄ as substrates for methanotrophs (Cui et al., 2021; Ji et al., 2018). To our knowledge, no studies have reported *in situ* field measurements over multiyear seasons of the effect of biochar addition on CH₄ uptake in forest soils. However, no significant effect of biochar addition on soil respiration was found over both short-term (49 days) incubation and long-term (15 month) *in situ* experiments in temperate forest soils (Burckman et al., 2015; Cui et al., 2021). Moreover, under free nutrient limitation conditions in the soil, the addition of biochar can enhance mycorrhizal colonization due to the increase of soil nutrient availability and potentially increased soil carbon sequestration via root-derived carbon transfer to the soil (Warnock et al., 2007; McCormack et al., 2013; Verma & Reddy, 2020). Further studies are needed to understand the impact of biochar addition on soil CH₄ uptake and soil respiration with the presence of ECM fungi in the long-term, which could change nutrient cycling.

2.2.3 Seasonal effect

A number of studies have reported a seasonal pattern of CH₄ uptake and soil respiration in temperate forest soils, with the highest rates during the growing season (May-October) and the lowest rates during winter (November-April) (Butterbach-Bahl and Papen, 2002; Borken and Beese, 2006; Borken et al., 2006; Wang, Yang and Zhang, 2006), which can be driven by

soil moisture or soil temperature. It was reported that soil CH₄ uptake was negatively correlated to soil moisture (Borken and Beese, 2006; Borken et al., 2006), while soil temperature had positive or no effect on soil CH₄ uptake in temperate forest soils (Borken et al., 2006; Ueyama et al., 2015; Yang, Wang and Xu, 2017). Changes in air temperature and precipitation due to climate change can also influence the species richness of ECM fungi in temperate forests according to a global meta-study (Tedersoo et al., 2012). However, no studies have investigated the potential long-term effect and underlying mechanism of the presence of ECM mycelium and biochar addition on net soil CH₄ uptake and soil respiration in temperate forests.

Therefore, to identify the short-term and long-term effects of ECM mycelium and biochar on soil net CH₄ uptake and soil respiration and their potential underlying abiotic drivers, we undertook a long-term (2012 to 2021) manipulation experiment in a temperate forest soil.

The hypotheses were:

1. The presence of ECM mycelium increases net soil CH₄ uptake during spring or earlier summer, but decreases soil CH₄ uptake during autumn and winter, because seasonal variations in temperature and nutrients change the ECM community structure.
2. The presence of ECM mycelium increases soil respiration during spring or earlier summer.
3. Biochar application does not have significant long-term (more than 3 years) effects on net soil CH₄ uptake, but can decrease soil CH₄ uptake in the short-term (1-3 years) because it increases the soil nutrient availability.
4. Biochar application does not have significant long-term (more than 3 years) effects on soil respiration, but decreases soil respiration in the short-term (1-3 years).
5. Soil CH₄ and CO₂ fluxes show larger net soil CH₄ uptake and soil respiration during the growing season and smaller soil CH₄ uptake and soil respiration during winter.

2.3 Methods

2.3.1 Study site

The field site was at Wheldrake Wood, a managed woodland on a mineral soil, located 11 km south-east of York, United Kingdom, at 53°54'48"N, 0°59'39"W (UK Grid Reference SE661468). Mean annual maximum and minimum temperatures were 13.6 °C and 5.7 °C, respectively,

and annual total precipitation was 603.2 mm (1981 to 2010, data from Church Fenton station, located around 16 km away from Wheldrake Wood, UK Met Office Library & Archive (www.metoffice.gov.uk)). The research area is dominated by Lodgepole pine (*Pinus contorta*) planted in 1993 (information from Forest Research, England), with a few naturally emerged Silver birch (*Betula pendula*) and immature Western hemlock (*Tsuga heterophylla*) trees. The soil is a well-draining, fine sandy gley podzol with a superficial organic layer (O horizon *ca.* 3 cm deep) overlaying a 3 cm deep A_h horizon (Heinemeyer *et al.*, 2007).

2.3.2 Experimental design

A long-term experiment spanning 8.5 years was carried out to determine soil surface CH₄ and CO₂ fluxes in response to ectomycorrhizal mycelium (ECM) and biochar (2012 - 2021) (Fig. 2.1). A randomised full-factorial block design was set up on 8th December 2011 with two parallel rows of plots and four contrasting treatments: with biochar addition and extraradical ECM (root exclusion) ('BSM'), with biochar addition and without extraradical ECM ('soil only', root and ECM exclusion) ('BS'), without biochar addition and with extraradical ECM ('XSM'), and without biochar addition and extraradical ECM ('XS'). For this purpose, 24 PVC collars (15 cm height, 10 cm inner diameter, 10.4 cm outer diameter), 6 replicates per treatment, were cut into the soil up to 13 cm depth, leaving about 2 cm collar above the soil surface. Each collar had three 4 cm × 4 cm windows evenly cut into their sides (top of windows 2.5 cm from the top of the collar) which were covered with either 41 µm nylon mesh enabling ingrowth of extraradical ECM hyphae but excluded roots or 1 µm mesh excluding both roots and extraradical ECM hyphae (Heinemeyer *et al.*, 2007). For each collar with biochar addition (from *Miscanthus* pyrolysed at 450°C, C: 67.22%, N: 0.45%, pH: 9.25, M450; BTG, Enschede, The Netherlands), the organic top 3 cm of soil (down to the Ah horizon) was removed, mixed with 9.4 g biochar at a rate of 12 t ha⁻¹ and then replaced. Biochar addition rate was based on the variety of biochar application rates and effects on CH₄ flux in published papers (Chan *et al.*, 2007; Warnock *et al.*, 2010; Jin, 2010). For each collar without biochar addition, the top 3 cm of the soil was similarly removed, mixed and replaced to mimic the same disturbance as for the treatment with biochar addition.

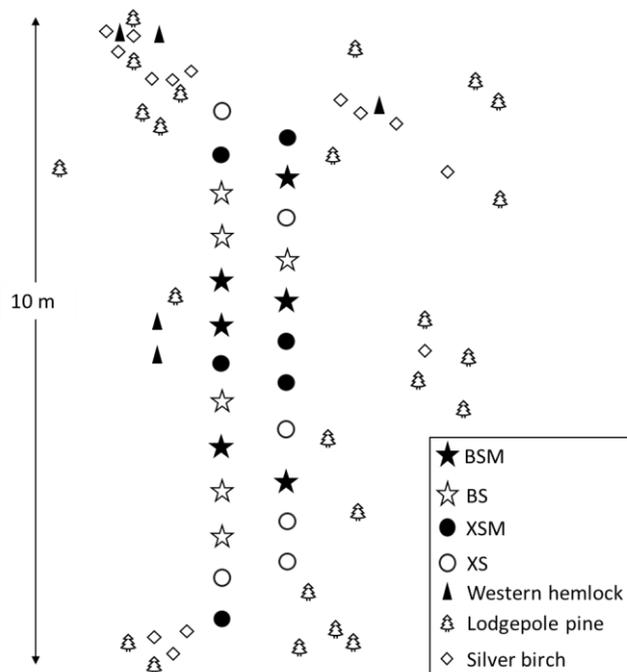


Figure 2. 1 Schematic diagram of the collars' placement. Approximate position of the trees is shown. Most Lodgepole pine (tree icons) and birch (diamonds) are ≥ 12 m in height. All Western hemlock (triangles) are ≤ 1.5 m. Distance between rows is approximately 1 m with about 0.5 m between collars. The abbreviation of treatment represents: BS is 1 μm mesh with biochar (light star), XS is 1 μm mesh without biochar (light circle), BSM is 41 μm mesh with biochar (black star) and XSM is 41 μm mesh without biochar (black circle).

2.3.3 Soil gas flux measurements and calculations

Soil surface CH_4 and CO_2 fluxes were measured 20 times *in situ* during September 2012 to May 2021. Fluxes of CH_4 and CO_2 were measured using the static chamber method (Fig. 2.2), which captured the change in CH_4 and CO_2 concentration in the headspace every second. A 10-cm diameter survey chamber (LI-8100-102, Li-Cor, Lincoln, Nebraska, USA) was placed over each PVC collar during measurement, forming an air-tight sealing around the outside of the collars with a rubber gasket. CO_2 and CH_4 fluxes were measured using a LI-8100 infra-red gas analyser (IRGA, Li-Cor, Lincoln, Nebraska, USA) and an Ultra-Portable Greenhouse Gas Analyser (UGGA, Los Gatos Research, Inc., Mountain View, CA, USA), respectively. The UGGA was put parallel to the IRGA, as it has a lower flow rate than the IRGA. During the flux measurements, each chamber was closed for 3 min, and with 30 seconds in between measurements to make sure CH_4 and CO_2 fluxes started again from *in situ* atmospheric concentrations by providing good ventilation. To determine the total volume of the gas enclosed by the gas flux set up, the chamber volume was measured from the distance between the soil surface inside the collar ($n=3$) to the top of the collar. The volume of the

Bev-A-Line Tubing (0.3 cm inner diameter, Cole-Parmer, UK) to connect the chamber and the gas analysers and the inner volume of the gas analysers were also determined.

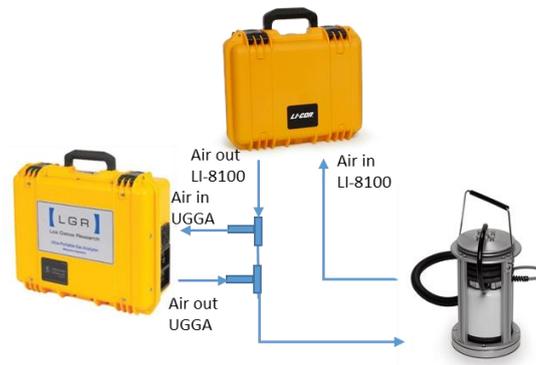


Figure 2. 2 Diagram of experimental setup on soil gas flux measurement.

Soil CH₄ and CO₂ fluxes were calculated from the raw data collected by the UGGA and LI-8100 using SoilFluxPro Software (v4.2.1; Li-COR, Lincoln, Nebraska). Soil CO₂ fluxes were measured by both instruments, but we used the CO₂ fluxes from the LI-8100 rather than the UGGA. Although CO₂ fluxes from both instruments showed a strong linear relationship (R²=0.87), the airstream was measured first by the LI-8100 and thus considered to provide a more accurate CO₂ flux data.

Gas flux was calculated using the following equation:

$$F = \left(\frac{dC}{dt}\right) \times \frac{PV}{ART}$$

where F is the flux of the particular gas, dC/dt is the change in concentration over time (ppm s⁻¹), P is atmospheric pressure, T is Kelvin temperature, R is the universal gas constant, A is the chamber surface area and V is the system volume. Flux units are reported in µg m⁻² h⁻¹ or mg m⁻² h⁻¹.

We calculated CH₄ and CO₂ fluxes using exponential regression of the concentration measurements obtained during each 3 min chamber closure. For both CH₄ and CO₂ fluxes, the first 30 s of each measurement were removed to allow for complete mixing of chamber air. To obtain a better fit of the regression, each exponential regression of CH₄ flux used a window length from 30-170 s and each exponential regression of CO₂ flux used a window length from 30-110 s. We removed CO₂ flux (2 of 480 measurements, 0.4% of the data) when an improper chamber closure with negative CO₂ flux or a sudden peak during measurement occurred. The

minimum detectable flux (MDF) was also calculated for both fluxes (Courtois et al., 2019). Consequently, we further removed CH₄ fluxes (2 of 480 measurements, 0.4% of the data) that did not meet the MDF standard. All other valid fluxes were kept regardless of R² of CH₄ and CO₂, because small flux rates tended to show lower R².

To compare the effect of biochar and ECM on cumulative CH₄ and CO₂ fluxes over different periods, we divided the entire study period into short-term 1-2 years (Sep 2012-Nov 2013), 2.5-3 years (May-Dec 2014), and longer-term 8.5-9.5 years (Jul 2020-May 2021) based on the longest successive measurement periods over time. And cumulative fluxes were calculated for each collar using the linear trapezoidal method.

2.3.4 Ancillary measurements

Soil temperature (at 2.5, 5 and 10 cm depth) and volumetric soil water content (0-6 cm depth) were measured by a hand-held Hanna temperature probe and ML2x theta probe (Delta-T Devices, Cambridge, UK), respectively. Soil temperature (n=1 at each depth) and soil water content (n=3) were measured both inside and outside each collar. Soil samples were collected three times during 2012-2021 (September of 2012, October of 2020 and May of 2021) at 0-10 cm soil depth to analyse ammonium (NH₄⁺-N) and nitrate (NO₃⁻-N) concentration. For each soil sample, we used 20 ml of 0.5M KCl to extract inorganic nitrogen from 2 g soil. All samples were shaken at 200 RPM for 1 hour on Orbital Shaking Platform (PSU-20i, Grant Instruments (Cambridge) Ltd, UK) and filtered over pre-rinsed GF/C (Glass Microfiber Filter Papers, WhatmanTM, Cytivia), and after extraction samples were measured by AA3 HR AutoAnalyzer (SEAL Analytical, Inc., UK). To investigate the potential environmental drivers of the gas fluxes, data for daily mean air temperature and daily precipitation from 2012 to 2021 were obtained at Cawood, located approximately 14.3 km southwest of the study site (UK Met Office Library & Archive (www.metoffice.gov.uk)).

2.3.5 Statistical analysis

All the statistical tests were performed in SPSS Statistics Software (Version 28; IBM Crop.). The gas flux and environmental data met the assumptions of homogeneity of variance (Levene's test) and normality (Kolmogorov-Smirnov test) and only CO₂ flux we used log transformation to reduce heteroscedasticity. Visual inspection of residual plots and normality test (Kolmogorov-Smirnov test) of the residuals did not reveal any obvious deviations from

homoscedasticity or normality (after log transformation). We performed a linear mixed effects analysis to assess the effects of the ECM and biochar treatments on the CH₄ and CO₂ fluxes and environmental variables (soil moisture, soil temperature at the 5 cm depth and inorganic nitrogen content) over time. For CH₄ and CO₂ fluxes and environmental variables, the fixed factors presence of ECM mycelium, biochar addition and time (and their interactions) and the random factor individual collar were included in the model. Due to the observed significant time × ECM and time × biochar interaction effects, a two-way Analysis of Variance (ANOVA) test was conducted to test for ECM and biochar effects on both CH₄ and CO₂ fluxes at individual sampling dates. To test the effect of ECM and biochar on cumulative CH₄ and CO₂ fluxes during each cumulative period, a two-way ANOVA test was performed. Correlations between CH₄ and CO₂ fluxes and soil moisture, soil temperature, initial CH₄ concentration, ammonium, nitrate and inorganic nitrogen concentration were analysed using Spearman's rank method.

2.4 Results

2.4.1 ECM effect on soil CH₄ uptake and respiration

Soil CH₄ uptake from the soil only (XS) and the presence of ECM mycelium treatments (XSM) over the measuring period were -88.84 ± 51.40 (mean \pm SD) and -74.63 ± 60.93 $\mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$, respectively (Fig. 2.3a). In the long-term (2012-2021), the presence of ECM did not have a significant effect on the soil CH₄ uptake ($P=0.228$), based on the linear mixed model (Table 2.1). However, there was a significant ECM × time interaction effect on soil CH₄ uptake ($P=0.003$). The presence of ECM mycelium had a fluctuating effect on net soil CH₄ uptake over the years, but the effect was not consistent (Fig. 2.3a). In September and October 2012, the presence of ECM mycelium resulted in lower soil net CH₄ uptake rates ($P=0.009$ and 0.024), but this effect reversed in December 2012 ($P=0.055$), showing higher CH₄ uptake in the presence of ECM mycelium. In May 2013, ECM mycelium again decreased soil net CH₄ uptake rates ($P=0.054$) and the opposite pattern was found in August 2013 ($P=0.058$). In 2014, the presence of ECM mycelium did not have a significant impact on soil CH₄ uptake. However, ECM mycelium presence resulted in a lower CH₄ uptake by the soil in August and November 2020 ($P=0.011$ and 0.038) compared to bulk soil treatment.

Soil CO₂ flux from the soil only (XS) and the presence of ECM mycelium (XSM) treatments over the measuring period were 211.68 ± 131.89 and 227.92 ± 176.72 mg CO₂ m⁻² h⁻¹, respectively (Fig. 2.3b). In the long term, based on a linear mixed model (Table 2.1), the presence of ECM did not show significant effect on soil respiration (P=0.096). However, there was a significant ECM × time interaction on the soil CO₂ flux (P=0.001). . Similar to soil CH₄ uptake, the effects of ECM presence on soil respiration were not consistent over the years (Fig. 2.3b). In October 2012 and May 2013, the presence of ECM significantly inhibited soil respiration (P= 0.004 and 0.056), but the direction of the ECM effect reversed in August 2013 (P= 0.001) with significantly larger CO₂ flux when ECM mycelium was present. However, ECM mycelium again tended to decrease soil respiration (P=0.083) in November 2013. During 2014 to 2021, 9 out of the 11 measurement dates did not present the effect of ECM mycelium on soil respiration, except for May 2014 and September 2020 (P=0.060 and 0.057), in which ECM mycelium tended to inhibit soil respiration.

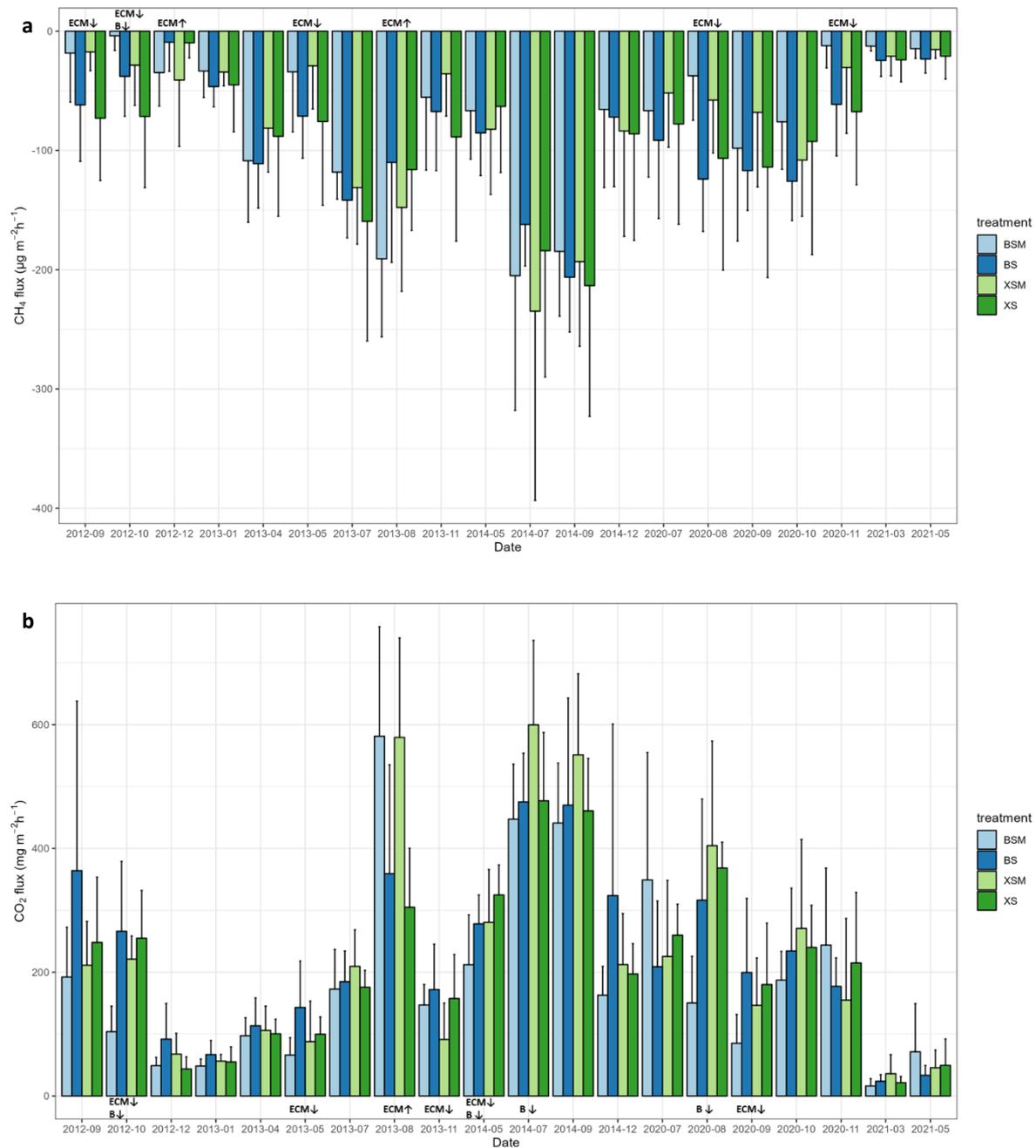


Figure 2. 3 Measurement of CH₄ (a) and CO₂ (b) fluxes in each treatment: BSM (biochar addition with ECM, light blue), BS (biochar addition without ECM, dark blue), XSM (ECM without biochar addition, light green) and XS (without ECM and biochar addition, dark green) during 2012 to 2021. Each sampling date represents CH₄ and CO₂ fluxes with standard deviation as error bars (n = 6 collars per treatment). Sampling dates were described as year- month (yyyy-mm). The trend of ECM and biochar effect on CH₄ and CO₂ fluxes during each sampling date was marked using ↓ for negative effect and ↑ for positive effect (P<0.1).

Table 2. 1 Linear mixed model results. Significant coefficients are highlighted in bold with the level of significance indicated: $p < 0.001$ (***), $p < 0.01$ (**), $p < 0.05$ (*), $p < 0.1$ (+). ECM represents ectomycorrhizal presence treatment and biochar represents biochar addition treatment.

Type III Tests of Fixed Effects					
Source	df	soil CH ₄ flux		soil CO ₂ flux	
		F	P	F	P
Intercept	1	181.51	<0.001 ***	15511.18	<0.001 ***
Date	19	41.05	<0.001 ***	133.84	<0.001 ***
ECM	1	1.55	0.228	2.96	0.096+
biochar	1	0.02	0.894	0.34	0.565
Date × ECM	19	2.93	0.003 **	3.66	0.001 ***
Date × biochar	19	1.09	0.397	1.79	0.071+
ECM × biochar	1	<0.01	0.950	1.53	0.225
Date × ECM × biochar	19	0.64	0.851	1.44	0.179

Since the establishment of the experiment, we studied the effect of ECM mycelium presence on cumulative CH₄ and CO₂ fluxes during short-term 1-2 years (Sep 2012-Nov 2013) and 2.5-3 years (May-Dec 2014) and longer-term 8.5-9.5 years (Jul 2020-May 2021) (Fig. 2.4). Similar to the effect of ECM mycelium on soil CH₄ uptake over the years, the impact of ECM mycelium on cumulative CH₄ uptake over the short-term and long-term was not consistent. We did not observe any significant effect of the ECM mycelium presence on cumulative CH₄ uptake during short-term 1-3 years (Sep 2012-Nov 2013 and May-Dec 2014) ($P=0.660$ and 0.846). However, the presence of ECM mycelium significantly decreased the cumulative CH₄ uptake during the long-term 8.5-9.5 years (Jul 2020-May 2021) ($P=0.041$). We did not observe any significant effect of the ECM mycelium presence on cumulative CO₂ flux during short-term and long-term over 1-9.5 years ($P=0.409$, 0.940 and 0.450).

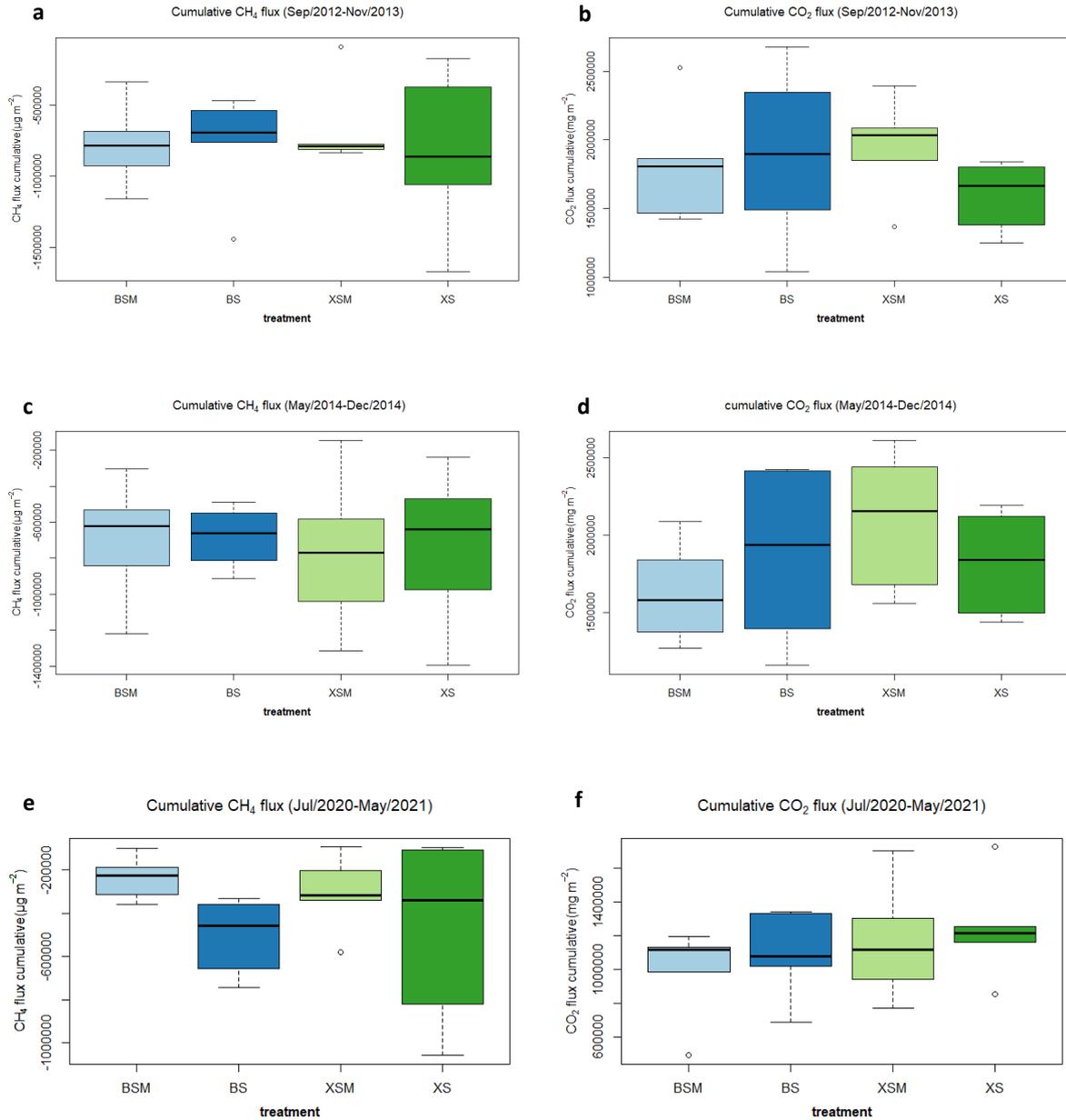


Figure 2. 4 Cumulative CH₄ and CO₂ flux of each treatment during September 2012 to November 2013 (a, b), May to December 2014 (c, d) and July 2020 to May 2021 (e, f). Treatments are shown as BSM (biochar addition with ECM, light blue), BS (biochar addition without ECM, dark blue), XSM (ECM without biochar addition, light green) and XS (without ECM and biochar addition, dark green).

2.4.2 Biochar effect on soil CH₄ uptake and respiration

Soil CH₄ uptake from biochar addition treatments with (BSM) and without ECM mycelium (BS) over the measuring period were -71.86 ± 61.76 and $-87.49 \pm 50.25 \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$, respectively (Fig. 2.3a). In the long-term, based on the linear mixed model (Table 2.1), biochar addition did not have a significant effect on soil CH₄ uptake ($P=0.894$) and no biochar \times time interaction

effect was observed ($P=0.397$). During the entire studying period (2012-2020), we only observed that biochar addition had a negative trend on soil CH_4 uptake in October 2012 ($P=0.080$) and 19 out of the 20 measurement dates did not present a significant effect of biochar addition on soil CH_4 uptake. We did not observe any significant effect of biochar addition on cumulative CH_4 uptake during short-term and long-term over 1-9.5 years ($P=0.918$, 0.652 and 0.873, Fig. 2.4).

Soil CO_2 flux from biochar addition treatments with (BSM) and without ECM mycelium (BS) over the measuring period were 191.22 ± 152.55 and 225.10 ± 131.65 $\text{mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$, respectively (Fig. 2.3b). In the long-term, based on the linear mixed model (Table 2.1), biochar addition did not have a significant effect on soil respiration ($P=0.565$). However, biochar \times time interaction tended to affect soil respiration ($P=0.071$). Biochar addition mostly had no significant effect on soil respiration during the entire measurement. In October 2012, biochar addition tended to decrease soil respiration ($P=0.095$) and this trend disappeared until 2014, which showed biochar addition reduced soil respiration again in May and July 2014 ($P=0.050$ and 0.090). During September 2014 to May 2021 (1 out of the 9 measurement dates), biochar addition exhibited a significant negative effect on soil respiration only in August 2020 ($P=0.007$). However, biochar addition did not show any significant effect on cumulative CO_2 flux during short-term and long-term over 1-9.5 years ($P=0.672$, 0.219 and 0.198, Fig. 2.4).

2.4.3 Seasonal effects on soil CH_4 uptake and respiration

Among all the collars, soil CH_4 uptake followed a seasonal trend over the study period with higher CH_4 uptake in summer (June to August, -125.73 ± 86.79 $\mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$) and autumn (September to November, -83.13 ± 77.98 $\mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$) and lower CH_4 uptake in winter (December to February, -46.95 ± 52.90 $\mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$) and spring (March to May, -52.89 ± 48.72 $\mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$). Based on the linear mixed model (Table 2.1), time and time \times ECM interaction showed a significant effect on soil CH_4 uptake ($P<0.001$ and $P=0.003$). Soil CH_4 uptake significantly changed over time in both soil with ECM mycelium treatment and soil only treatment ($P<0.001$). Similar to soil CH_4 uptake, soil CO_2 flux followed a seasonal trend among all the collars over the study period with higher soil respiration in summer and autumn (342.47 ± 180.57 and 238.83 ± 151.23 $\text{mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$) and lower soil respiration in winter and spring (115.56 ± 119.29 and 110.68 ± 100.55 $\text{mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$). Based on the linear mixed model (Table 2.1), time and time \times ECM interaction also showed a significant effect on soil

respiration ($P < 0.001$ and $P = 0.001$). Soil respiration significantly changed over time in both soil with the ECM mycelium treatment and the soil only treatment ($P < 0.001$).

Soil ammonium and nitrate concentrations were measured three times during 2012-2021 at 0-10 cm soil depth (Fig. 2.5). In all the treatments, the mean value of ammonium, nitrate and inorganic nitrogen concentrations were 4.98 ± 5.81 , 5.83 ± 6.95 and 10.82 ± 10.96 mg/N kg dry soil, respectively. We did not observe any effect of ECM presence and biochar addition on ammonium, nitrate and inorganic nitrogen concentrations ($P > 0.05$). Soil temperature at 5 cm depth, soil moisture and initial atmospheric CH₄ concentration of each treatment were measured during the entire study (Fig. 2.6). The mean soil temperature of all the treatments over the years was 10.8°C, ranging from 2.3 to 17.7°C, and soil moisture ranged from 3.82 to 42.40 %vol with a mean of 16.89 %vol. The presence of ECM and biochar addition did not show any significant effect on soil moisture, soil temperature and initial CH₄ concentration ($P > 0.05$). When the effect of ECM mycelium presence and biochar addition on soil CH₄ uptake and soil respiration occurred at specific sampling dates (see results above), we did not observe any significant difference in ammonium, nitrate, inorganic nitrogen concentrations, soil moisture and soil temperature between treatments ($P > 0.05$). This suggested that the potential drivers behind the treatment effect could be some other biotic or abiotic factors that we did not measure. In addition, the effect of ECM mycelium presence on soil CH₄ uptake and soil respiration took place at various soil moisture (4.59-42.4%vol) and soil temperature (2.3-17.7°C) conditions.

Based on the linear mixed models, time showed significant effects on ammonium, nitrate, inorganic nitrogen concentrations, soil moisture and soil temperature during the entire study ($P < 0.001$). We analysed the soil CH₄ and CO₂ fluxes of each treatment during the measurement period to determine the potential environmental drivers (Table 2.2). Among all the treatments, soil net CH₄ uptake increased with decreasing soil moisture ($P < 0.001$) and increasing soil temperature and soil CO₂ flux ($P < 0.001$). Only the treatment with biochar addition and ECM mycelium presence (BSM) showed significant negative correlation between soil CH₄ uptake and nitrate and inorganic nitrogen concentrations ($P = 0.033$ and 0.048). Interestingly, we found soil CH₄ uptake also showed a significant negative correlation with initial atmospheric CH₄ concentration ($P < 0.05$), except for the treatment with ECM mycelium presence without biochar addition (XSM) ($P = 0.188$). Soil respiration significantly increased

with soil temperature in all the treatments ($P < 0.001$), but had no relationship with soil moisture ($P > 0.05$).

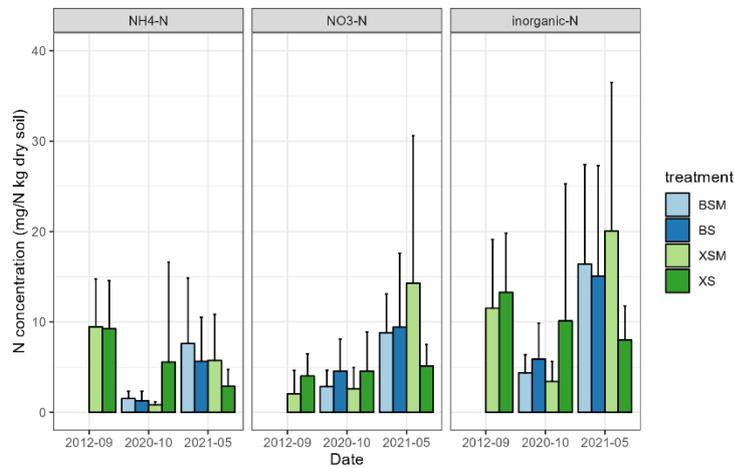


Figure 2. 5 Ammonium, nitrate and inorganic nitrogen concentrations of each treatment ($n = 6$ collars per treatment) during September 2012 to May 2015. Treatments are shown as BSM (biochar addition with ECM, light blue), BS (biochar addition without ECM, dark blue), XSM (ECM without biochar addition, light green) and XS (without ECM and biochar addition, dark green).

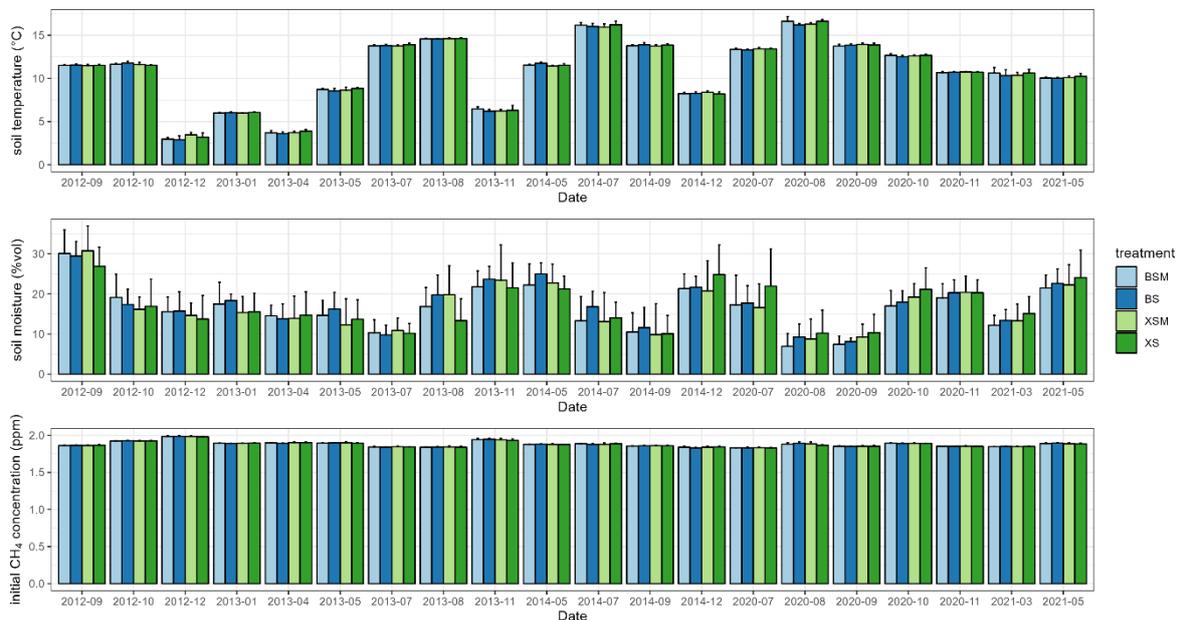


Figure 2. 6 Soil temperature at 5 cm depth, soil moisture and initial atmospheric CH₄ concentration of each treatment ($n = 6$ collars per treatment) during 2012 to 2021. Treatments are shown as BSM (biochar addition with ECM, light blue), BS (biochar addition without ECM, dark blue), XSM (ECM without biochar addition, light green) and XS (without ECM and biochar addition, dark green).

Table 2. 2 Spearman's Rank-Order Correlation between soil CH₄ and CO₂ fluxes and environmental variables of each treatment throughout the entire study's data collection. Treatments are shown as BSM (biochar addition with ECM), BS (biochar addition without ECM), XSM (ECM without biochar addition) and XS (without ECM and biochar addition). Significant coefficients are highlighted in bold with the level of significance indicated: p < 0.001 (***), p < 0.01 (**), p < 0.05 (*), p < 0.1 (+).

Spearman's Rank-Order Correlation															
Treatment	soil gas flux	soil moisture		soil temperature		initial CH ₄ concentration		Ammonium-N		Nitrate-N		inorganic-N		soil CO ₂ flux	
		r _s (N)	P	r _s (N)	P	r _s (N)	P	r _s (N)	P	r _s (N)	P	r _s (N)	P	r _s (N)	P
BSM	CH ₄ flux	0.346(118)	<0.001***	-0.372(118)	<0.001***	0.213(118)	0.020*	0.364(10)	0.245	0.615(10)	0.033*	0.580(10)	0.048*	-0.472(117)	<0.001***
	CO ₂ flux	0.046(117)	0.622	0.551(117)	<0.001***	-	-	-0.209(9)	0.537	-0.436(9)	0.180	-0.518(9)	0.102	-	-
BS	CH ₄ flux	0.325(118)	<0.001***	-0.530(118)	<0.001***	0.252(118)	0.005**	0.538(10)	0.071+	0.000(10)	1.000	0.252(10)	0.430	-0.465(118)	<0.001***
	CO ₂ flux	0.059(118)	0.524	0.575(118)	<0.001***	-	-	-0.559(10)	0.059+	0.112(10)	0.729	-0.203(10)	0.527	-	-
XSM	CH ₄ flux	0.403(117)	<0.001***	-0.390(117)	<0.001***	0.121(117)	0.188	0.451(16)	0.060+	-0.009(16)	0.971	0.337(16)	0.171	-0.509(117)	<0.001***
	CO ₂ flux	-0.063(118)	0.491	0.695(118)	<0.001***	-	-	-0.137(16)	0.587	-0.280(16)	0.260	-0.228(16)	0.363	-	-
XS	CH ₄ flux	0.534(117)	<0.001***	-0.402(117)	<0.001***	0.259(117)	0.005**	0.096(16)	0.705	0.015(16)	0.951	0.053(16)	0.836	-0.461(116)	<0.001***
	CO ₂ flux	-0.055(117)	0.549	0.692(117)	<0.001***	-	-	-0.082(16)	0.748	-0.170(16)	0.499	-0.220(16)	0.381	-	-

2.5 Discussion

2.5.1 ECM mycelium effect on soil CH₄ uptake

The soil CH₄ uptake rates in the treatments of soil only (XS) and ECM mycelium presence (XSM) during springtime were -55.44 ± 55.74 and -45.78 ± 44.09 $\mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$, respectively, which was comparable to the study measured at the same research site in May 2009 (-34.08 and -47.36 $\mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$) (Subke et al., 2018). During 13 out of the 20 measurement dates, we did not observe a significant ECM mycelium effect on net soil CH₄ uptake. However, the effect of ECM mycelium presence on net soil CH₄ uptake fluctuated between positive and negative several times during the short-term 1-2 years (Sep 2012-Nov 2013), whilst during the long-term 8.5-9.5 years (Jul 2020-May 2021) cumulative CH₄ uptake was around 1.5 times significantly lower when ECM mycelium was present. The results were more complicated compared to what Subke et al. (2018) have found at the same research site during the short-term five weeks (May-June 2009), reporting about 1.4 times higher net soil CH₄ uptake in ECM mycelium presence treatment than those in the soil only treatment. In contrast, we found the presence of ECM mycelium tended to inhibit soil CH₄ uptake once in spring (May 2013, $P=0.054$) over 8.5 years of measurement.

We did not observe differences in ammonium, nitrate and inorganic nitrogen concentrations between the only soil with no ECM treatment (XS) and ECM fungi presence treatment (XSM) when the ECM mycelium effect on soil CH₄ uptake occurred. However, the treatment with biochar addition and ECM mycelium presence (BSM) showed a significant negative correlation between soil CH₄ uptake and nitrate and inorganic nitrogen concentrations on three occasions during the measurements ($P=0.033$ and 0.048 , Table 2.2). Similar results have shown that NO_3^- addition to forest soils exhibited a stronger inhibitory effect on CH₄ oxidation than NH_4^+ (Wang and Ineson, 2003; Reay and Nedwell, 2004; Mochizuki, Koba and Yoh, 2012). It was suggested that NO_3^- as an oxidant for denitrifiers that cannot only outcompete methanogens for substrate, which inhibits CH₄ production (Bodelier and Steenbergh, 2014), but also added NO_3^- and nitrite (NO_2^-) produced by nitrification or denitrification processes are probably toxic to methanotrophs (Schnell and King, 1994; Wang and Ineson, 2003).

The presence of soil fungi can change soil chemical conditions, such as soil pH, the availability of organic carbon, N and P (Smith and Read, 2008) and thus potentially alter the environment for soil bacteria. A theory was proposed that the growth of the methanotrophic community may benefit from the presence of ECM mycelium by providing more labile carbon and thus increasing soil CH₄ uptake (Subke et al., 2018). However, the effect of ECM fungi presence on methanotrophs community and soil CH₄ uptake may be more complicated. First, the amount and type of exudation (such as organic acids) released from ECM fungi were influenced by the tree species (Martin et al., 2008) and the fungal species (Sandnes et al., 2005). Second, ECM fungi community exhibited large temporal variation, which could be caused by the difference tolerance to changing soil resource availability and temperature (Courty et al., 2008). Third, methanotrophs were likely to live in areas with low carbon and nutrient cycling rates in temperate forests (Burke et al., 2012). It was reported that although the relative abundance of methanotrophs increased with the higher available carbon and mineral nitrogen in a mixed temperate forest floor, the soil CH₄ uptake decreased, which may be explained by the increasing activity of methanogens or the alternative use of other carbon substrates rather than the atmospheric CH₄ by methanotrophs (Jilkova et al., 2016). Furthermore, we did not observe any difference in soil temperature and soil moisture between the soil only treatment (XS) and ECM fungi presence treatment (XSM) when the ECM mycelium effect on soil CH₄ uptake occurred. Considering the abiotic factors we have measured in this study, it was still difficult to explain the fluctuation effect of ECM mycelium presence on soil CH₄ uptake during short-term 1-2 years and the more consistent effect during long-term 8.5-9.5 years. Further studies are required to understand the impact of the temporal relationship between ECM fungi and methanotrophs community and nutrient cycling rates on soil CH₄ uptake in temperate forest soils.

2.5.2 ECM mycelium effect on soil respiration

The soil respiration rates in the treatments of soil only (XS) and ECM mycelium presence (XSM) during springtime were 119.17 ± 113.34 and 111.22 ± 103.37 mg CO₂ m⁻² h⁻¹, respectively, which was comparable to the study measured at the same research site in May 2009 (85.55 and 102.98 mg CO₂ m⁻² h⁻¹) (Subke et al., 2018).

Similar to soil CH₄ uptake, the effect of ECM mycelium presence on net soil respiration was not consistent. The presence of ECM mycelium exhibited both positive and negative effects on soil respiration during the short-term 1-2 years (Sep 2012-Nov 2013), which was strongly consistent with the direction of ECM fungi effect on soil CH₄ uptake. However, we did not observe a significant ECM mycelium effect on cumulative CO₂ flux over 1-9.5 years. Due to the measurement limitation of soil nutrient availability in this experiment, we only measured ammonium, nitrate and inorganic nitrogen concentrations on three occasions and the ECM mycelium effect on soil respiration did not occur during those occasions. Additionally, we did not observe any significant correlation between soil respiration and inorganic N content (including NH₄⁺-N and NO₃⁻-N) at our research site (Table 2.2), which may be due to the exclusion of roots in our experiment. It was indicated that adding NH₄NO₃ can reduce the annual soil respiration rates by approximately 24% in temperate forests, which was mainly due to the inhibition of fine roots production (Zhang et al., 2019).

One study using the same mesh-collar approach in the woodland area of this experiment, reported that ECM mycelium increased soil respiration by 22% in the first month after experimental installation and contributed to around 25% of soil CO₂ flux during short-term three campaigns (June-December 2005) (Heinemeyer et al., 2007). However, Subke et al. (2018) did not find a significant difference in soil respiration between the soil only treatment and ECM mycelium treatment after 12 months of collar insertion at the same research site, which was explained by the small amount of mycorrhizal biomass inside the ECM mycelium treatment. It was reported that although the newly grown (12-16 months before harvest) ECM mycelium increased soil respiration, ECM mycelium biomass was not correlated to its cumulative respiration during autumn in temperate forest soils, which may be due to the turnover of ECM mycelium (Neumann and Matzner, 2014). The estimates of ECM mycelium turnover can vary from months to several years depending on nutrient availability (Cairney, 2012). The seasonal variation of ECM fungal respiration in temperate forest soils has been reported as a peak in summer following an initial increase in spring, and a decrease in autumn (Yan et al., 2019). The temporal trend of ECM fungal respiration could be explained by the variation in the supply of aboveground photosynthates to

the rhizosphere (Subke et al., 2011), as ECM fungi strongly rely on carbohydrates from host plants (Yan et al., 2019). Compared to soil bacteria, the diversity of soil fungi exhibited larger seasonal fluctuation and the seasonal abundance and diversity of soil fungi were correlated to soil fertility in temperate forests (Shigyo, Umeki and Hirao, 2019), which might explain the fluctuation effect of ECM mycelium presence on soil respiration we observed at our research site. Further studies are required to determine the temporal variation effect of ectomycorrhizal biomass on soil respiration and the response of ECM fungi to belowground carbon and nitrogen cycling in a long-term (more than 3 years) in temperate forest ecosystems, which can help to improve the estimation of global soil respiration data considering mycorrhizal contribution (Bond-Lamberty and Thomson, 2010).

2.5.3 Biochar effect on soil CH₄ uptake and respiration

Our results showed the addition of biochar did not have significant effects on soil CH₄ uptake and cumulative CH₄ flux during short-term and long-term over 1-9.5 years. Similar results were also reported in biochar-amended temperate forest soils where soil CH₄ flux was not affected during 4 months of lab incubation and 12 months of field study (Malghani, Gleixner and Trumbore, 2013; Sackett et al., 2015). Biochar application into forest soils can potentially alter soil physical (e.g., soil bulk density, soil porosity and soil water holding capacity), chemical (e.g., soil pH, soil organic carbon pools and soil nutrient availability) and microbial properties (e.g., microbial biomass and microbial community structure) (Li et al., 2018). The lack of biochar addition effect on soil CH₄ uptake in our study might be explained by several reasons. According to a meta-analysis, the effect of biochar addition on soil CH₄ flux mostly depended on biochar pH and soil texture (He et al., 2017). Biochar pH may change the ratio of soil methanogenic to methanotrophic abundance (Anders et al., 2013), however, the soil pH data we collected in May 2021 did not show a significant difference between biochar and non-biochar addition treatments ($P > 0.05$) with the biochar pH of 9.25. It was reported that biochar addition to coarse soils can improve soil aeration, which is favorable for methanotrophs communities (Van Zwieten et al., 2009). However, the forest soils are classified as sandy fine-textured soils at our research site. The effect of aeration could be inhibited as the porous structure of

biochar maybe be filled with a fine silt fraction (He et al., 2017). Furthermore, the type of biochar production can also influence the response of soil CH₄ flux to biochar addition (Malghani, Gleixner and Trumbore, 2013). The type of biochar we applied to the soils was obtained from Miscanthus and slowly pyrolysed at 450°C. It was shown that slow pyrolysis (temperature ≤ 500°C) of biochar addition did not affect CH₄ uptake in temperate forest soils, whilst the amendment of biochar produced by hydrothermal carbonization (low temperature, high pressure) from the same feedstock material switched soil from CH₄ uptake to emission, which might be explained by the increasing anaerobicity (Malghani, Gleixner and Trumbore, 2013).

The effect of biochar addition on soil respiration was not consistent during the entire study period. Four out of the 20 measurement dates showed biochar addition tended to inhibit soil respiration ($P < 0.10$), but no biochar effect was presented during the other measurement dates. Additionally, biochar amendment did not show any significant effect on cumulative CO₂ flux during short-term and long-term over 1-9.5 years. Similar to our results, other studies also reported no significant change in soil respiration in temperate forest soils over 4 months to 4 years after biochar addition (Malghani, Gleixner and Trumbore, 2013; Sackett et al., 2015; Cui et al., 2021). We did not observe any significant differences in soil moisture and soil temperature between biochar and non-biochar addition treatments when the effect of biochar addition on soil CO₂ flux occurred in our study. It was reported that the response of soil CO₂ flux to biochar addition also depended on biochar properties (He et al., 2017). The type of biochar (from Miscanthus pyrolysed at 450°C) we applied to the forest soils at our research site may explain the decrease in soil respiration. A study has shown that higher relative concentrations of toxic compounds may be found in high pyrolysis temperature biochars (Nakajima et al., 2007), which may inhibit soil microbial biomass and activity and thus reduce soil respiration rates. Pokharel et al. (2018) observed that pine sawdust biochar produced at 300°C did not affect soil CO₂ emission, while biochar produced at 550°C can reduce about 16.4% of cumulative CO₂ emissions in temperate forest soils compared to the control, which was due to lower microbial biomass and enzyme activities. However, the inconsistent biochar addition effect on soil respiration over the entire study period may be due to the fine sandy soil texture at our research

site. The results from a meta-analysis showed that compared to coarse and medium texture, no significant effect of biochar addition on soil CO₂ flux was found in fine texture soils (He et al., 2017). Further studies are needed to understand the effect of biochar type on soil respiration at different soil textures in temperate forest soils.

2.5.4 Seasonal effects on soil CH₄ uptake and respiration

Soil CH₄ uptake and soil respiration rates were significantly correlated with each other in all the treatments during 2012 to 2021, and seasonal patterns were found with the highest rates during June to November and the lowest rates during December to May. The results were consistent with other studies (Butterbach-Bahl and Papen, 2002; Borken and Beese, 2006; Borken et al., 2006; Wang, Yang and Zhang, 2006; Yamulki and Morison, 2017). During the entire study period, all treatments showed a significant negative correlation between soil CH₄ uptake and soil moisture. It was reported that the increased soil moisture (60-100% water-filled pore space) can decrease air-filled pore space and hence limit the diffusion of atmospheric CH₄ through the soil to methanotrophs (Castro et al., 1995; Reay, Smith and Hewitt, 2007). Soil CH₄ uptake significantly increased with soil temperature over the 8.5 years of measurements at our research site, which may be explained by the temperature sensitivity of the underlying enzymatic process (Steinkamp, Butterbach-Bahl and Papen, 2001; Luo et al., 2013). Surprisingly, we found three treatments showed a significant negative correlation between soil CH₄ uptake and initial atmospheric CH₄ concentration over the long-term 8.5 years of measurement (Table 2.2). However, it was reported that soil CH₄ uptake was positively related to initial atmospheric CH₄ concentration at the same research site during the short-term five weeks (May-June 2009) (Subke et al., 2018). Our interpretation is that soil temperature may be the limiting environmental factor on soil CH₄ uptake. Soil temperature in our study over the long-term 8.5 years ranged from 2.3 to 17.7°C and was significantly negatively correlated to atmospheric initial CH₄ concentration ($r_s(478)=-0.448$, $P<0.001$). However, the soil temperature ranged only from 10 to 12°C during the short-term five weeks (May-June 2009) (Subke et al., 2018). It was indicated that when soils became warmer and drier, soil CH₄ uptake may be controlled by the CH₄ diffusion ability rather

than temperature which is correlated to methanotrophic activity in mineral soils (Bowden, Newkirk and Rullo, 1998; Luo et al., 2013).

In consistent with other studies, we observed soil respiration increased with soil temperature, due to higher microbial activity (Wu et al., 2011; Oertel et al., 2016). However, there was no relationship between soil respiration and soil moisture over the 8.5 years of measurements. Higher soil moisture can limit substrate availability and thus inhibit soil respiration by reducing the activity of soil microorganisms (Davidson, Janssens and Luo, 2006; Han and Jin, 2018). The response of soil respiration to soil moisture can be observed more clearly in dry environments (Wu et al., 2011). It was reported that soil moisture significantly affected soil respiration in temperate forest soils only when soil temperature at 10 cm depth (Q_{10}) increased with soil moisture, but soil temperature became the only factor affecting soil respiration when Q_{10} decreased with soil moisture (Wang, Yang and Zhang, 2006). In our study, soil moisture was significantly negatively correlated to soil temperature at 5 cm depth ($r_s(478)=-0.292$, $P<0.001$) during the entire measurement period, which may lead to soil temperature as the main driver of the seasonal variations of soil respiration.

2.6 Conclusion

We studied the effect of ECM mycelium presence and biochar addition on net soil CH₄ uptake and soil respiration during the long-term measurement of 8.5 years in temperate, coniferous mineral forest soils. Compared to bare soils, soil CH₄ uptake and CO₂ fluxes fluctuated when ECM mycelium were present during the short-term 1-2 years. In addition, the presence of ECM mycelium only significantly decreased the cumulative CH₄ uptake during the long-term 8.5-9.5 years (Jul 2020-May 2021). Considering the abiotic factors we have measured in this study, it was still difficult to explain the inconsistent effect of ECM mycelium presence on soil CH₄ uptake and respiration over the 8.5 years of study. Further studies are required to understand the temporal variations of ECM fungi, and their relationship with methanotrophs community and belowground carbon and nitrogen cycling rates in a long-term (more than 3 years) in temperate forests. Biochar addition did not show any significant effect on soil CH₄ uptake and mostly no effect on soil respiration over the entire study, which

suggested that the biochar type we applied to the forest soils at our research site did not show any beneficial effect on soil CH₄ uptake. Compared to ECM mycelium and biochar effect, soil CH₄ uptake and soil respiration exhibited stronger seasonal patterns over the years. Soil CH₄ uptake increased with increasing soil temperature and decreasing soil moisture, while soil respiration only positively correlated to soil temperature.

3. Methane and Carbon Dioxide Exchange from Tree Stems and Forest Soils in a Temperate Upland Forest

3.1 Abstract

Mineral soils in temperate forests are important biological CH₄ sinks. However, recent studies reported tree stem CH₄ emissions in temperate upland forests may offset soil CH₄ sink and contribute to the global CH₄ budget. In order to determine the role of tree stem CH₄ flux in forests, tree stem and soil CH₄ and CO₂ fluxes from English oak (*Quercus robur*) and Japanese larch (*Larix kaempferi*) were measured during the spring and summer period of 2020 in a temperate upland forest. Both oak and larch trees showed stem CH₄ uptake and emission at 45 cm above the soil, and mean tree stem CH₄ fluxes were -0.43 ± 2.01 and 0.39 ± 1.99 $\mu\text{g m}^{-2}$ stem surface h⁻¹, respectively. The mineral forest soil acted as a net CH₄ sink with -21.84 ± 17.59 and -59.70 ± 33.72 $\mu\text{g m}^{-2}$ soil surface h⁻¹ at the oak and larch sites, respectively. Tree stem CH₄ flux did not show a significant effect on tree species (P=0.256) and seasonal difference (P=0.888) based on the linear mixed model. However, tree stem CO₂ flux of both tree species exhibited a seasonal pattern, which was positively correlated to soil temperature (P<0.001) and air temperature (P<0.01) and negatively correlated to soil moisture (P<0.01). Tree stem CH₄ and CO₂ fluxes of English oak and Japanese larch taken at 45 cm and 130 cm above the soil surface once in August, showed no significant height effect on stem CH₄ (P=0.525) and CO₂ (P=0.805) fluxes. Contrary to stem CO₂ flux from the oak and larch, we found large daytime and intra-specific variations in the tree stem CH₄ fluxes showing both uptake and emission. These results indicate that the underlying mechanism and pathway of tree stem CH₄ exchange is complicated in temperate upland forests.

3.2 Introduction

Methane (CH₄) and carbon dioxide (CO₂) are two important greenhouse gas (GHG) playing a vital role in global climate change (Manabe, 2019). Mineral forest soils are one of the most significant biological sinks for CH₄ (Smith et al., 2000; Le Mer and Roger, 2001; Dutaur and Verchot, 2007), while soil CO₂ flux contributes to the second

largest flux in the carbon budget of forest ecosystems (Peng, Thomas and Tian, 2008). However, the global CH₄ budget is still highly uncertain, which requires an improving estimation of the global CH₄ sources from all ecosystems (Saunois et al. 2020). In recent years, more studies have reported *in situ* measurements of tree stem CH₄ in upland forests (Wang et al., 2016, 2017; Machacova et al., 2016; Warner et al., 2017; Pitz and Megonigal, 2017; Maier et al., 2018; Pitz et al., 2018; Barba, Poyatos and Vargas, 2019; Moldaschl et al., 2021; Barba et al., 2021). However, because of the large spatial and temporal variability in tree stem CH₄ emissions in upland forests and limited knowledge of the underlying mechanisms, upscaling these CH₄ stem fluxes to the landscape scale is difficult (Covey and Megonigal, 2019; Barba et al., 2019, 2021).

Basically, there are mainly two assumptions about the mechanisms of CH₄ emissions from living tree stems, which depend on the forest ecosystem (either wetland or mineral forest soils) and tree species (containing dry and dense or wet and porous wood) (Yip et al., 2018). There is evidence that in floodplain and wetland temperate forests, CH₄ is biological produced in anoxic saturated soils or dissolved in groundwater, and then absorbed by roots and transported in stems through intercellular spaces and aerenchyma tissue via the transpiration stream, and finally diffused by tree stem to the atmosphere (Terazawa et al., 2007, 2015; Sakabe et al., 2021). However, in well-drained temperate forests on mineral soils where soil is a net CH₄ sink, it was believed that tree stem CH₄ was produced biologically *in situ* inside the heartwood (Covey et al., 2012; Wang et al., 2016, 2017; Yip et al., 2018; Barba et al., 2021). In contrast, the mechanisms of stem CO₂ flux releasing to the atmosphere have been widely studied (Etzold et al., 2013; Teskey and McGuire, 2007; Teskey et al., 2017). Tree stem CO₂ flux can originate from respiring cells in the stems and roots and/or from the rhizosphere via xylem transportation (Teskey et al., 2008). The presence of chlorophyll inside bark tissues has suggested stem internal fixing of CO₂ released by respiratory activity (Pfanzen and Aschan, 2001; Pilarski and Tokarz, 2006; Berveiller, Kierzkowski and Damesin, 2007). However, more studies are needed to understand the diffusion and internal production of stem CO₂ flux in different wood tissues among various tree species and stem heights under different temperatures and water content of the stems (Teskey et al., 2017).

Studies have shown large differences in tree stem CH₄ emissions between tree species in temperate upland forests, which may be explained by the species-specific differences in disease resistance and wood anatomy (Warner et al., 2017; Pitz and Megonigal, 2017; Pitz et al., 2018). It was suggested that the difference in wood anatomy between gymnosperm and angiosperm (tracheid vs vessels) may have an impact on gas diffusion or microbial colonization, and thus affect stem CH₄ emissions (Barba et al., 2018). To our knowledge, almost all studies of tree stem CH₄ flux in temperate upland forests have been carried out on deciduous broadleaved tree species. So far tree stem CH₄ flux has only been determined on one deciduous coniferous tree species (*Larix gmelinii* (Ruprecht) Kuzeneva)) in temperate upland forests, but stem CH₄ flux was undetectable (Wang et al., 2016). However, in boreal forests, studies have found CH₄ uptake from the branch of Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) (Sundqvist et al., 2012) and CH₄ emissions from Scots pine tree stem and shoots (Machacova et al., 2016). In addition, a recent study further presented Norway spruce shoots have the ability to produce and consume CH₄ via *in situ* field measurements and novel metagenomic tools (Putkinen et al., 2021). Tree stem CO₂ flux can also vary among different tree species due to the variability in wood tissue respiration and radial diffusion rate through the xylem to the atmosphere (Teskey et al., 2017).

Variations in stem CH₄ fluxes have been observed at different stem heights in temperate upland forests. It was reported that stem CH₄ fluxes decreased with increasing stem height, which may support the assumption of the belowground source of CH₄ and CO₂, transported via the stem transpiration stream (Pitz and Megonigal, 2017; Barba, Poyatos and Vargas, 2019). The *in situ* measurements of tree stem CH₄ and CO₂ fluxes at different stem heights may help to understand the underlying mechanism of tree stem CH₄ and CO₂ sources.

Furthermore, it was suggested that studying diurnal and seasonal patterns of tree stem CH₄ and CO₂ fluxes along with the potential environmental drivers (e.g. soil moisture, temperature and sap flow rate) can help to understand the source and pathway of stem CH₄ and CO₂ fluxes (Barba et al., 2019, 2021; Covey and Megonigal, 2019). However, a recent study found large variations of tree stem CH₄ and CO₂ fluxes

between individual trees and large temporal variations of tree stem fluxes within a given tree stem (Flanagan et al., 2021). But the current knowledge of this source of large variation was still limited. It was suggested that the biological heartwood *in situ* CH₄ production and its complicated pathway of CH₄ diffusion between the stem and the atmosphere may contribute to this variation (Wang et al., 2016; Flanagan et al., 2021).

To unravel the seasonal difference of tree stem CH₄ and CO₂ fluxes within and between tree species, this research aimed to determine the variations of tree stem CH₄ and CO₂ fluxes within two species of contrasting anatomy, English oak (*Quercus robur*, deciduous broadleaf) and Japanese larch (*Larix kaempferi*, deciduous conifer) from two sites during a growing season from a temperate, managed forest on mineral soil in the UK.

The hypotheses examined are:

1. Tree stem CH₄ and CO₂ fluxes are larger from English oak than those from Japanese larch due to different wood anatomy, while soil acts as a net CH₄ sink at both research sites.
2. Tree stem CH₄ and CO₂ fluxes decrease with increasing stem height of both tree species.
3. Tree stem and soil CH₄ and CO₂ fluxes exhibit seasonal variations, which are caused by environmental drivers (such as soil moisture, soil temperature and air temperature).

3.3 Methods

3.3.1 Study site

The research was carried out in Wheldrake Wood, a managed woodland on a mineral soil, located 11 km south-east of York, United Kingdom, at 53°54'48"N, 0°59'39"W (UK Grid Reference SE661468). The soil is a well-draining, sandy loam with a superficial organic layer (O horizon *ca.* 3 cm deep) overlaying a 3 cm deep A_n horizon (Heinemeyer *et al.*, 2007). Mean annual maximum and minimum temperatures were 13.6 °C and 5.7 °C, respectively, and the annual total precipitation was 603.2 mm (1981 to 2010, data from Church Fenton station, located around 16 km away from Wheldrake Wood, UK Met Office Library & Archive (www.metoffice.gov.uk)). In two research plots with

an area of 1439 and 5251 m², respectively, either dominated by the deciduous broadleaf species English oak (*Quercus robur*) or by the deciduous coniferous tree species Japanese larch (*Larix kaempferi*), twelve trees were selected for this study (Table 3.1).

Table 3. 1 The characteristics of selected tree species (n=12 trees per tree species) at Wheldrake Wood

Tree species	Common name	DBH ^a (cm) Mean ± SD	Tree height (m) Mean ± SD	Tree anatomy	Year planted
<i>Quercus robur</i>	English Oak	32.7 ± 6.7	21.0 ± 4.4	ring-porous	1961
<i>Larix kaempferi</i>	Japanese larch	18.7 ± 3.4	18.1 ± 2.6	non-porous	1997

^aDBH: Diameter at breast height

3.3.2 Experimental design

To compare the effect of tree species on stem CH₄ and CO₂ fluxes, fluxes of CH₄ and CO₂ were measured *in situ* from tree stems of English oak and Japanese larch (n=12 per species) at 45 cm above the soil surface, and from soil close to each tree stand (n=3 per tree) during March, June and August 2020. To identify the effect of stemheight on tree stem CH₄ and CO₂ fluxes, gas flux measurements were also taken from 130 cm above the soil surface in August 2020. To assess the diurnal pattern of tree stem CH₄ and CO₂ fluxes, we took gas flux measurements from each tree species (n=12 per species) at 45 cm above the soil surface on three occasions during 9:30-16:30 in June 2020. Due to Covid-19 restrictions, we did not take measurements of tree stem and soil CH₄ and CO₂ fluxes during April to May 2020 and the study of stemheight effect on tree stem CH₄ and CO₂ fluxes was only tested once on both tree species in August 2020.

3.3.3 Chamber design and installation

Tree stem and soil fluxes were measured using a static chamber approach. The rigid tree stem chamber (Fig. 3.1) was adapted from (Machacova et al., 2021) and consisted of a transparent plastic food container (Lock & Lock, 470 ml, 13.7 cm × 10.4 cm). The bottom of the container was cut off and a 2.5 cm wide flange of acrylic plastic (thickness of 5 mm; Hindleys, UK) was attached around the container. The flange was covered with a 10 mm thick adhesive neoprene rubber sponge (RS Components Ltd., UK). Before attaching the rigid chambers to tree stems, sealant (Zero VOC sealant,

Ecomerchant, UK) was tested as producing no CH₄ and CO₂ emissions (Appendix 1A) and was applied to fill stem cracks and the gap between the rigid chambers and stems. The rigid tree stem chambers were permanently attached to the trees, two weeks prior to the experimental period at 45 cm above the soil in February 2020, and at 130 cm above the soil in July 2020.

To compare tree stem fluxes with soil fluxes, 3 PVC collars (10 cm height, 20 cm inner diameter, 20.4 cm outer diameter) were located 1-1.5 m from each tree stem (n=36 collars per tree species) and inserted into the litter/soil layer leaving about 5 cm collar above the soil surface. A 20-cm diameter survey chamber (LI-8100-103, Li-Cor, Lincoln, Nebraska, USA) was placed over each PVC collar during measurement, forming an air-tight sealing around the outside of collars with a rubber gasket (Fig. 3.1). Soil collars were first installed 2 weeks before flux measurements and remained in place throughout the experimental period.

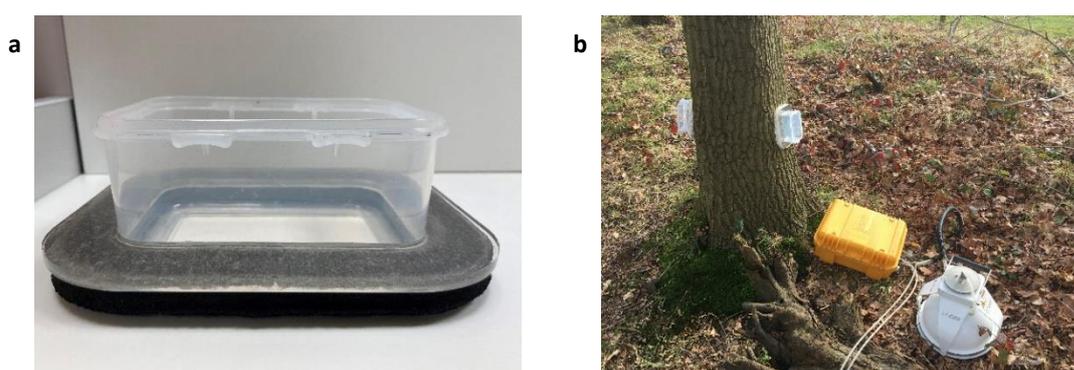


Figure 3. 1 Tree stem and soil gas fluxes were measured by rigid tree stem chambers (a) and a 20-cm diameter survey chamber (b), respectively.

3.3.4 Tree stem and soil flux measurements

To measure fluxes from a larger surface area of the wider oak tree stems, each English oak tree was installed with two rigid chambers north and south of the stems at each stem height due to the larger stem diameters and interconnected with 220 cm Bev-A-Line Tubing (0.3 cm inner diameter, Cole-Parmer, UK) into one flow-through chamber system. Only one chamber at each stem height was installed on each Japanese larch due to the smaller stem diameter (Fig. 3.2). During tree stem flux measurements, the lid was attached to the stem chamber to form a gas-tight seal. The lid contained an inlet and outlet (Quick Connect Bulkhead Plug x .165" OD, The West Group Ltd, UK)

which were attached to an Ultra-Portable Greenhouse Gas Analyser (UGGA, Los Gatos Research, Inc., Mountain View, CA, USA) with 440 cm of bev-a-line tubing in a closed loop (Fig. 3.3). During measurements, each tree stem gas fluxes were determined over a 6-min period with the instrument measuring concentration at a rate of 1Hz. Before each measurement, initial CH₄ and CO₂ fluxes were checked to make sure they return to *in situ* atmospheric concentrations.

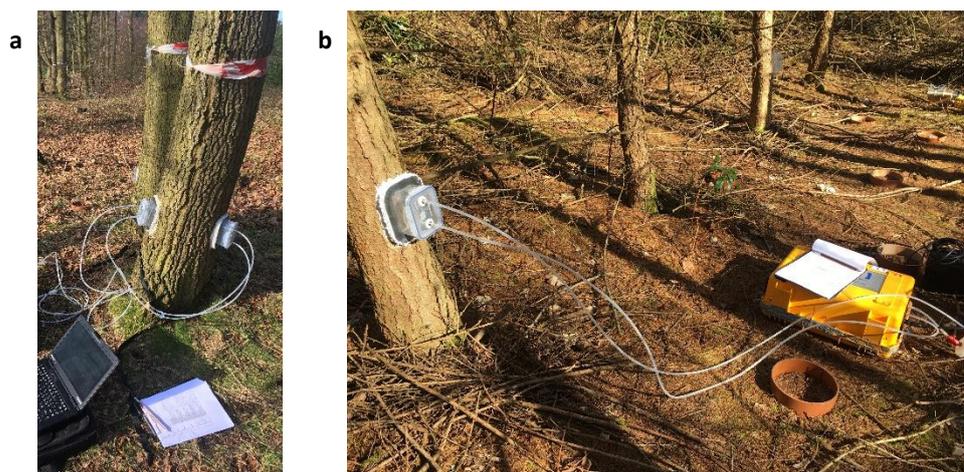


Figure 3. 2 Rigid tree stem chambers on English oak (a) and Japanese larch (b) stems at 45 cm above the soil surface during gas flux measurements (with lid attached).

Soil CH₄ and CO₂ fluxes were measured by paralleling the UGGA to a LI-8100 infra-red gas analyser (IRGA, Li-Cor, Lincoln, Nebraska, USA), as LI-8100 controls the opening and closing of the survey chamber (Fig. 3.3). During the measurements, each soil chamber was closed for 3 min with 30 seconds in between measurements. To determine the volume of the gas enclosed by the soil chamber and experimental setup, distances from soil surface to the top of the collar were measured inside the collar (n=3). The volume of the Bev-A-Line Tubing (0.3 cm inner diameter, Cole-Parmer, UK) to connect the chamber and the gas analysers and the inner volume of the gas analysers were also determined.

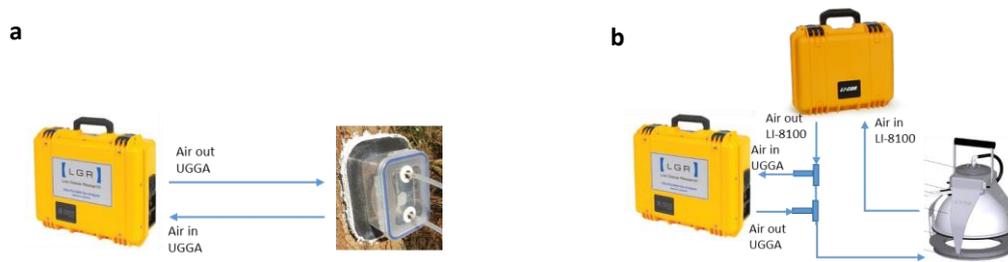


Figure 3. 3 Diagram of experimental setup on tree stem (a) and soil (b) gas flux measurement.

3.3.5 Stem and soil gas flux calculations

Tree stem and soil CH₄ and CO₂ fluxes were calculated from the 1Hz concentration data collected by the UGGA and LI-8100 (soil CO₂ fluxes only) instruments using SoilFluxPro software (v4.2.1; Li-COR, Lincoln, Nebraska). Soil CO₂ fluxes were obtained from both the UGGA and LI-8100, and we selected to use the soil CO₂ fluxes from LI-8100 rather than from the UGGA. As CO₂ fluxes from both instruments showed a strong linear relationship ($y=0.9136x + 9.2573$, $R^2=0.99$) and the airstream was measured first by the LI-8100, this may provide a more accurate CO₂ flux data.

Gas flux was calculated using the following equation:

$$F = \left(\frac{dC}{dt}\right) \times \frac{PV}{ART}$$

where F is the flux of the particular gas, dC/dt is the change in concentration over time (ppm s⁻¹), P is atmospheric pressure, T is Kelvin temperature, R is the universal gas constant, A is the chamber surface area and V is the system volume. Flux units are reported in μg m⁻² h⁻¹ or mg m⁻² h⁻¹.

We calculated tree stem and soil CH₄ and CO₂ fluxes using linear regression of the concentration measurements obtained during each 6 and 3 min chamber closure, respectively. The first 40 s (tree stem fluxes) or 30 s (soil fluxes) were discarded when the air in the system was mixing immediately after chamber closure. To obtain a better fit of the regression, each linear regression of tree stem and soil CH₄ flux used the window length from 40-300 s and 30-170 s, respectively, and each linear regression of tree stem and soil CO₂ flux used the window length from 40-160 s and 30-110 s, respectively. For quality control of gas fluxes, we firstly checked for leakage issues during each flux measurement and both CH₄ and CO₂ fluxes were removed when the

fluctuation of CO₂ concentration was observed. We removed tree stem CO₂ flux (51 of 168 measurements, 30.36% of the data) and tree stem CH₄ flux (51 of 168 measurements, 30.36% of the data) due to apparent leakage issues (Appendix 1B). We removed soil CO₂ flux (3 of 216 measurements, 1.39% of the data) and soil CH₄ flux (2 of 216 measurements, 0.93% of the data). Then we checked if both CH₄ and CO₂ flux measurements were above the minimum detectable flux (MDF) (Courtois et al., 2019), which removed a further tree stem CH₄ flux (4 of 168 measurements, 2.38% of the data) and soil CH₄ flux (1 of 216 measurements, 0.46% of the data) that did not meet the MDF standard. All other valid fluxes were kept regardless of R² of CH₄ and CO₂, because small flux rates tended to show lower R².

In order to compare gas fluxes between tree stems and soils at each of the two research sites, we estimated CH₄ and CO₂ tree stem fluxes expressed per m² soil surface. The total stem flux of each tree was determined individually during the measuring period. Firstly, we calculated tree surface area assuming a cone shape with tree DBH as the base of the cone and tree height as the cone height. Then multiplied tree surface area with tree stem gas fluxes (at 45 cm height) assuming the measurements were representative of the entire tree stem (Flanagan et al., 2021), and finally multiplied with tree density at each site to estimate the upscaling site level.

3.3.6 Environmental measurements

Air temperature inside and outside the rigid chamber was continuously measured every hour by ibuttons (Ibuttons, DS1922L Thermochron Data Logger, Measurement Systems Ltd, UK) positioned inside the chamber (attached to a box wrapped with foil to shade) and outside the chamber (attached to the lid) during measurements. Soil temperature (at 10 cm depth) and volumetric soil water content (0-6 cm depth) were measured immediately after a soil flux measurement by a hand-held Hanna temperature probe and ML2x theta probe (Delta-T Devices, Cambridge, UK), respectively. Soil temperature (n=3) and soil water content (n=3) were measured both inside and outside each PVC collar. At each of the two sites, data loggers (GP1, Delta-T Devices, Cambridge, UK) were installed to measure air temperature (10 cm above the soil, in shade), soil moisture (0-6 cm depth) and soil temperature (10 cm depth) continuously every four hours throughout the experiment (since June 2020).

The diameter (at 45, 130 and 200 cm above the soil) and height of each tree were measured using a measuring tape and a clinometer. To capture canopy openness during the growing season, we took circular hemispherical photographs under a clear sky using an iPhone (SE, 2020) equipped with 180° Fisheye Lens (Viga Europe) at a fixed height of 130 cm at the end of the experiment in September. For each tree, we took two pictures aligning the smartphone with north-south and with east-west by a compass, and always using the automatic exposure. Canopy openness was analysed by the software Gap Light Analyzer (GLA, Version 2.0, Simon Fraser University, Burnaby, British Columbia, and the Institute of Ecosystem Studies, Millbrook, New York).

3.3.7 Statistical analysis

All the statistical tests were performed in SPSS Statistics Software (Version 28; IBM Corp.). The gas flux and environmental data met the assumptions of homogeneity of variance (Levene's test) and normality (Kolmogorov-Smirnov test). Visual inspection of residual plots and normality test (Kolmogorov-Smirnov test) of the residuals did not reveal any obvious deviations from homoscedasticity or normality. We performed linear mixed effects analysis to assess the effects of the tree species on tree stem (at 45 cm height only) and soil CH₄ and CO₂ fluxes and soil temperature and moisture over time. For tree stem and soil CH₄ and CO₂ fluxes, the fixed factors tree species and time (and their interactions) and the random factor individual tree were included in the models. All pairwise comparison with Bonferroni correction of time was applied in the linear mixed model. Due to the observed significant tree species × time interaction effect, an Independent T test or a Mann-Whitney U test was conducted on both tree stem and soil CH₄ and CO₂ fluxes at each measuring time, in order to test the effect of tree species on tree stem and soil gas fluxes, and soil temperature and moisture. Correlations between tree stem (at 45 cm height only) and soil CH₄ and CO₂ fluxes, and soil moisture, soil temperature, air temperature, DBH, canopy openness and initial atmospheric CH₄ concentration were analysed using the Spearman's rank method. To study the effect of stem height and tree species on tree stem gas fluxes in August 2020, a two-way Analysis of Variance (ANOVA) was conducted on both tree stem CH₄ and CO₂ fluxes.

3.4 Results

3.4.1 Tree species and seasonal effects on tree stem and soil gas fluxes

During the measurement period, tree stem CH₄ flux of English oak and Japanese larch trees was -0.43 ± 2.01 (mean \pm SD) and 0.39 ± 1.99 $\mu\text{g m}^{-2}$ stem surface h⁻¹, respectively. Both tree species showed a large variation within individual trees during measurement occasions with both stem CH₄ uptake and emission, varying from -6.77 to 2.38 $\mu\text{g m}^{-2}$ stem surface h⁻¹ for English oak trees and -4.66 to 4.14 $\mu\text{g m}^{-2}$ stem surface h⁻¹ for Japanese larch trees (Fig.3.4). Based on the linear mixed model (Table 3.2), we did not observe a significant tree species or seasonal effect on tree stem CH₄ flux or a significant tree species \times season interaction. However, tree stem CO₂ fluxes significantly changed over the season ($P < 0.001$), and there was also a significant tree species and season interaction ($P = 0.002$). The tree stem CO₂ flux was significantly larger in Japanese larch trees in March and June ($P < 0.001$ and $P < 0.01$) but not in August. English oak tree stem CO₂ flux significantly increased with time, while Japanese larch tree stem CO₂ flux was significantly lower in spring (March) than in summer (June and August).

Table 3. 2 Linear mixed model results of tree species effects and seasonal variations of tree stem CH₄ and CO₂ fluxes. Significant coefficients are highlighted in bold with the level of significance indicated: $p < 0.001$ (***) , $p < 0.01$ (**).

Source	Type III Tests of Fixed Effects					
	df	tree stem CH ₄ flux		tree stem CO ₂ flux		
		F	P	F	P	
Intercept	1	<0.01	0.955	81.01	<0.001***	
month	2	0.12	0.888	25.78	<0.001***	
tree species	1	1.36	0.256	2.69	0.116	
month \times tree species	2	1.45	0.247	7.77	0.002***	

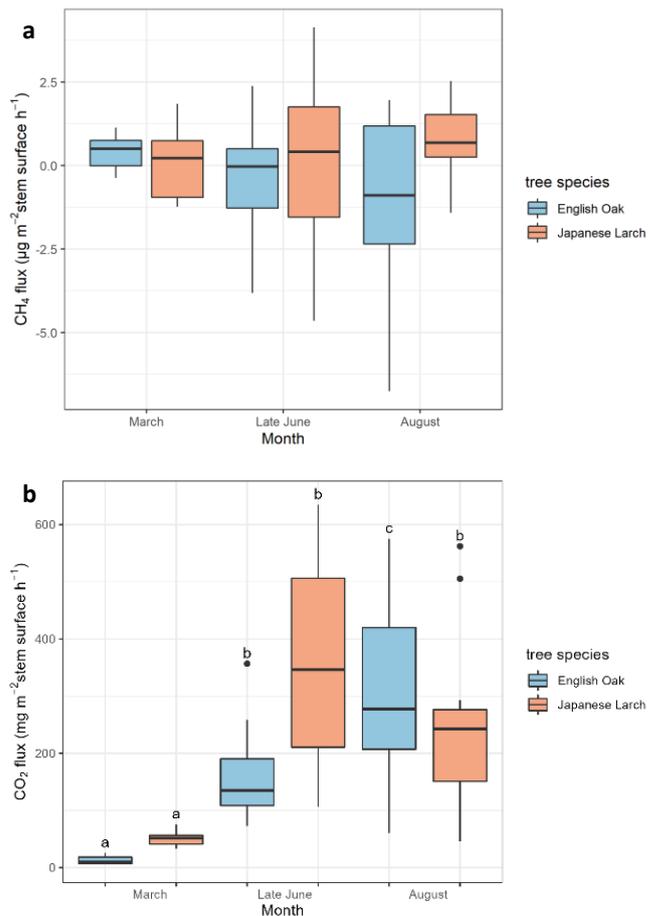


Figure 3. 4 Seasonal measurements of English oak (blue) and Japanese larch (red) tree stem CH₄ (a) and CO₂ (b) fluxes at 45 cm height in 2020 (n=12 individual trees per species per measurement). For all boxplots in this study, the black line in the box represents the median and the interquartile range box represents the middle 50% of the data. The whiskers extend from either side of the box. The whiskers represent the ranges for the bottom 25% and the top 25% of the data values, excluding outliers. The outliers are shown in black dots. Different letters indicate significant differences over time for each of the tree species (P<0.05). A positive flux indicates emission and a negative indicates uptake.

Compared to tree stem CH₄ flux, mineral forest soil is a net CH₄ sink with -21.84 ± 17.59 and -59.70 ± 33.72 µg m⁻² soil surface h⁻¹ at oak and larch sites, respectively (Fig. 3.5). Based on the linear mixed model (Table 3.3), we observed a significant effect of seasonal variation and tree species on soil CH₄ and CO₂ fluxes, and there was also a significant tree species and season interaction on soil CH₄ flux (P<0.001). Soil CH₄ uptake was significantly higher at the Japanese larch site than at the English oak site during each month's measurement (P<0.001), and soil CH₄ uptake significantly increased over time during the growing season at both study sites (P<0.001). Soil respiration was significantly lower at the Japanese larch site compared to the English

oak site during the study ($P=0.004$), and significantly higher soil respiration was found in summer (June and August) than in spring (March) at both study sites.

Table 3. 3 Linear mixed model results of tree species effects and seasonal variations of soil CH₄ and CO₂ fluxes. Significant coefficients are highlighted in bold with the level of significance indicated: $p < 0.001$ (***), $p < 0.01$ (**).

Type III Tests of Fixed Effects					
Source	df	soil CH ₄ flux		soil CO ₂ flux	
		F	P	F	P
Intercept	1	291.97	<0.001***	413.87	<0.001***
month	2	116.79	<0.001***	83.97	<0.001***
tree species	1	62.93	<0.001***	10.27	0.004**
month × tree species	2	21.44	<0.001***	1.51	0.231

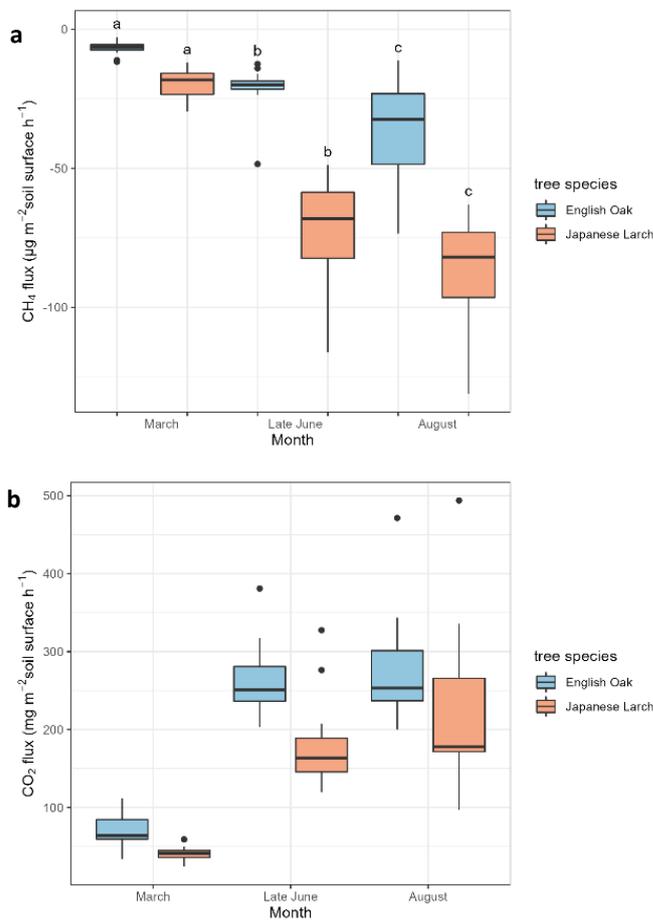


Figure 3. 5 Seasonal measurements of English oak (blue) and Japanese larch (red) soil CH₄ (a) and CO₂ (b) fluxes in 2020 (n=12 individual trees per species per measurement). Different letters indicate significant differences over time for each of the tree species ($P < 0.05$).

We observed a significant tree site, seasonal changes and tree site \times season interaction effect on soil moisture at 0-6 cm depth and soil temperature at 10 cm depth throughout the study period ($P < 0.001$). The soil moisture content was significantly higher in the English oak site than in the Japanese larch site during the entire study ($P < 0.01$) and soil moisture significantly decreased over time during the growing season at both study sites ($P < 0.001$, Fig. 3.6). The soil temperature did not significantly differ between the two sites, except for June when the soil temperature was significantly higher at the Japanese larch site ($P < 0.001$). Soil temperature significantly increased over time during the growing season at the English oak site ($P < 0.001$), while at the Japanese larch site, soil temperature was significantly higher in summer (June and August) than in spring (March) ($P < 0.001$).

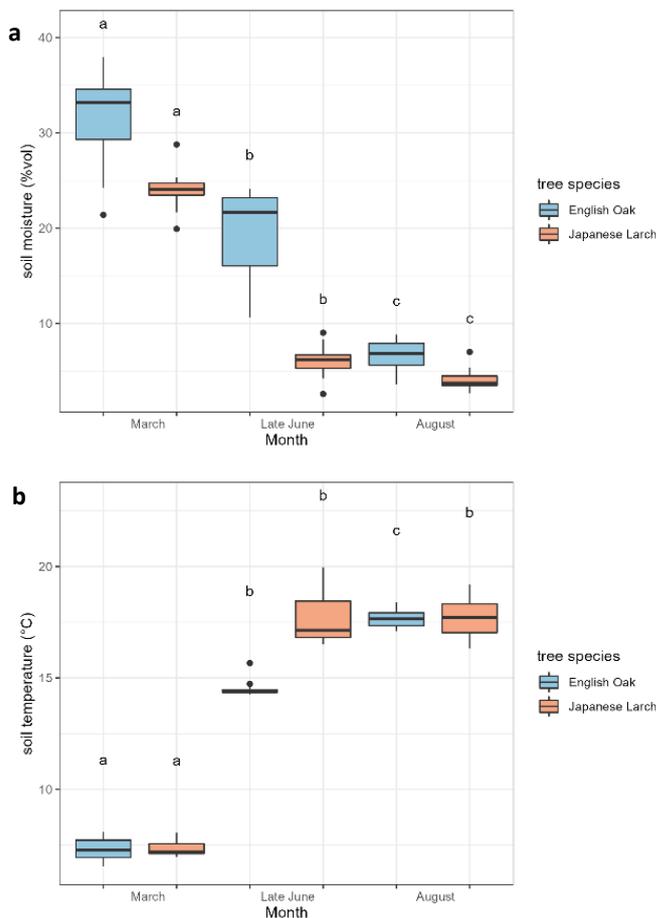


Figure 3. 6 Volumetric soil moisture content at 0-6 cm depth (a) and soil temperature at 10 cm depth (b) at study sites of English oak (blue) and Japanese larch (red) in 2020 (n=12 individual trees per species per measurement). Different letters indicate significant differences over time for each of the tree species ($P < 0.05$).

3.4.2 Effect of stem height

We measured tree stem CH₄ and CO₂ fluxes at 45 cm and 130 cm above the soil in August. Contrasting with the soil CH₄ uptake in August (Fig. 3.7), the tree stems showed mostly CH₄ emissions. Tree stem CH₄ flux of English oak was -0.44 ± 3.00 and $1.24 \pm 1.69 \mu\text{g m}^{-2}$ stem surface h⁻¹ at 45 cm and 130 cm, respectively, and tree stem CH₄ flux of Japanese larch was 1.30 ± 0.85 and $0.71 \pm 2.33 \mu\text{g m}^{-2}$ stem surface h⁻¹ at 45 cm and 130 cm, respectively (Fig. 3.7). The results showed no height, tree species and height \times tree species interaction effect on tree stem CH₄ flux ($P_{\text{height}}=0.525$, $P_{\text{species}}=0.483$, $P_{\text{height} \times \text{species}}=0.192$). We did not find a height effect on tree stem CO₂ flux either ($P=0.805$), but English oak (351.97 ± 150.84 and $304.38 \pm 173.27 \text{ mg m}^{-2}$ stem surface h⁻¹ at 45 cm and 130 cm) showed significantly larger stem CO₂ fluxes than those from Japanese larch (192.84 ± 109.92 and $211.78 \pm 133.36 \text{ mg m}^{-2}$ stem surface h⁻¹ at 45 cm and 130 cm) ($P=0.039$).

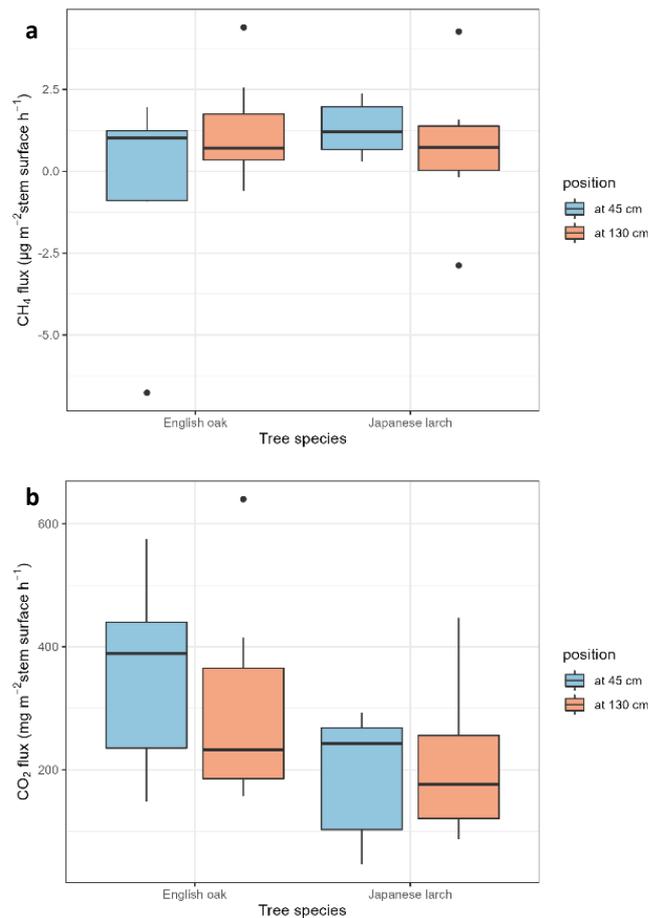


Figure 3. 7 Measurements of English oak (blue) and Japanese larch (red) tree stem CH₄ (a) and CO₂ (b) fluxes at 45 cm and 130 cm above the surface in August 2020 (n=7 individual trees for English oak and n=6 individual trees for Japanese larch).

3.4.3 Potential drivers of tree stem gas fluxes

We analysed the measurements of CH₄ and CO₂ fluxes from tree stems only at 45 cm height and soils collected during the entire study period to determine the potential environmental drivers (Table 3.4). Tree stem CH₄ fluxes of both tree species did not correlate significantly with soil moisture, soil temperature, DBH, canopy openness, initial CH₄ concentration, stem CO₂ flux and soil CH₄ flux, but we observed a positive relationship between air temperature and tree stem CH₄ flux for the larch (P=0.081). Both oak and larch tree stem CO₂ flux exhibited significant correlations with soil moisture, soil temperature, air temperature and soil CO₂ flux (P<0.01). At both English oak and Japanese larch study sites, soil CH₄ uptake and soil respiration presented significant positive correlations with soil temperature and air temperature (P<0.001), and a significant negative correlation with soil moisture (P<0.001). We also found a

significant positive correlation between soil CH₄ uptake and soil respiration at both sites ($P < 0.001$), and a negative correlation between soil CH₄ uptake and initial atmospheric CH₄ concentration at the English oak site ($P < 0.001$).

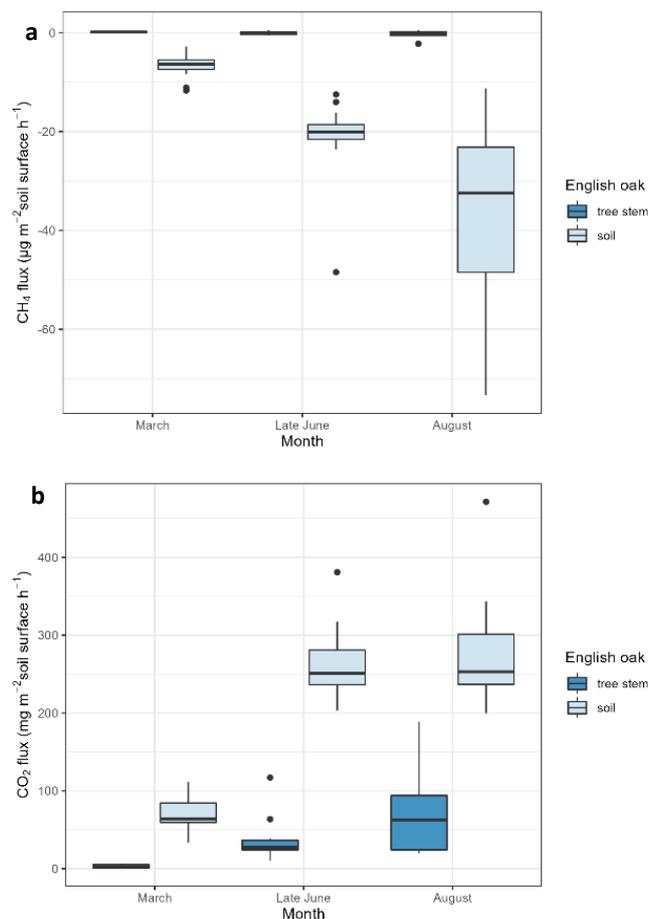
Table 3. 4 Spearman's Rank-Order Correlation between tree stem (only at 45 cm height) and soil CH₄ and CO₂ fluxes and environmental variables throughout the entire study's data collection. Significant coefficients are highlighted in bold with the level of significance indicated: p < 0.001 (***), p < 0.01 (**), p < 0.05 (*), p < 0.1 (+).

		Spearman's Rank-Order Correlation																	
		soil moisture		soil temperature		air temperature		DBH		canopy openness		initial CH ₄ concentration		stem CO ₂ flux		soil CH ₄ flux		soil CO ₂ flux	
		r _s (N)	P	r _s (N)	P	r _s (N)	P	r _s (N)	P	r _s (N)	P	r _s (N)	P	r _s (N)	P	r _s (N)	P	r _s (N)	P
English oak	stem CH ₄ flux	0.157 (24)	0.444	0.062 (24)	0.762	0.171 (33)	0.326	0.188 (33)	0.27 9	-0.017 (33)	0.92 3	-0.054 (33)	0.759	-0.128 (33)	0.464	0.187 (24)	0.360	-	-
	stem CO ₂ flux	0.683 (24)	<0.001** *	0.796 (34)	<0.001** *	0.662 (33)	<0.001** *	0.224 (33)	0.19 6	-0.002 (33)	0.99 1	-	-	-	-	-	-	0.722 (24)	<0.001** *
	soil CH ₄ flux	0.843 (34)	<0.001** *	0.814 (34)	<0.001** *	0.855 (34)	<0.001** *	-	-	-	-	0.847 (34)	<0.001** *	-	-	-	-	-0.801 (34)	<0.001** *
	soil CO ₂ flux	0.659 (34)	<0.001** *	0.739 (34)	<0.001** *	0.714 (34)	<0.001** *	-	-	-	-	-	-	0.722 (24)	<0.001** *	0.801 (34)	<0.001** *	-	-
Japanese larch	stem CH ₄ flux	0.015 (30)	0.933	0.161 (30)	0.379	0.266 (42)	0.081+	0.015 (42)	0.92 2	0.056 (42)	0.71 6	0.062 (42)	0.691	0.102 (42)	0.510	0.078 (30)	0.670	-	-
	stem CO ₂ flux	0.455 (32)	0.007**	0.544 (32)	<0.001** *	0.403 (44)	0.005**	0.050 (44)	0.74 1	0.069 (44)	0.64 8	-	-	-	-	-	-	0.492 (32)	0.003**
	soil CH ₄ flux	0.664 (34)	<0.001** *	0.689 (34)	<0.001** *	0.815 (34)	<0.001** *	-	-	-	-	-0.232 (34)	0.173	-	-	-	-	-0.708 (34)	<0.001** *
	soil CO ₂ flux	0.805 (34)	<0.001** *	0.821 (36)	<0.001** *	0.776 (34)	<0.001** *	-	-	-	-	-	-	0.492 (32)	0.003**	0.708 (34)	<0.001** *	-	-

- variables not analysed

3.4.4 Tree stem and soil gas flux comparison at site level

The mineral forest soils at these two study sites showed only CH₄ uptake, whereas both English oak and Japanese larch tree stems showed both CH₄ uptake and emission within individual trees on different measurement occasions. After converting tree stem CH₄ and CO₂ flux per stem surface into per soil surface, English oak tree stem CH₄ flux ranged from -2.22 to 0.57 $\mu\text{g m}^{-2}$ soil surface h⁻¹, and Japanese larch tree stem CH₄ flux ranged from -3.71 to 3.91 $\mu\text{g m}^{-2}$ soil surface h⁻¹ during the measuring period (Fig. 3.8). These estimated oak tree stem CH₄ emissions offset soil CH₄ uptake by approximately 1.63% in March, while larch tree stem CH₄ emissions offset soil CH₄ uptake by approximately 0.42% and 0.81% in June and August, respectively. At the English oak site, soil respiration (33.48-471.38 mg m⁻² soil surface h⁻¹) was higher than converted tree stem CO₂ flux (1.02-111.59 mg m⁻² soil surface h⁻¹) during the measuring period. In contrast to the oak site, at the Japanese larch site due to larger tree stem CO₂ emissions and tree density, soil respiration and converted tree stem CO₂ flux were comparable in March and August and tree stem CO₂ flux (314.78 ± 192.03 mg m⁻² soil surface h⁻¹) was even larger than soil respiration (181.50 ± 62.25 mg m⁻² soil surface h⁻¹) in June.



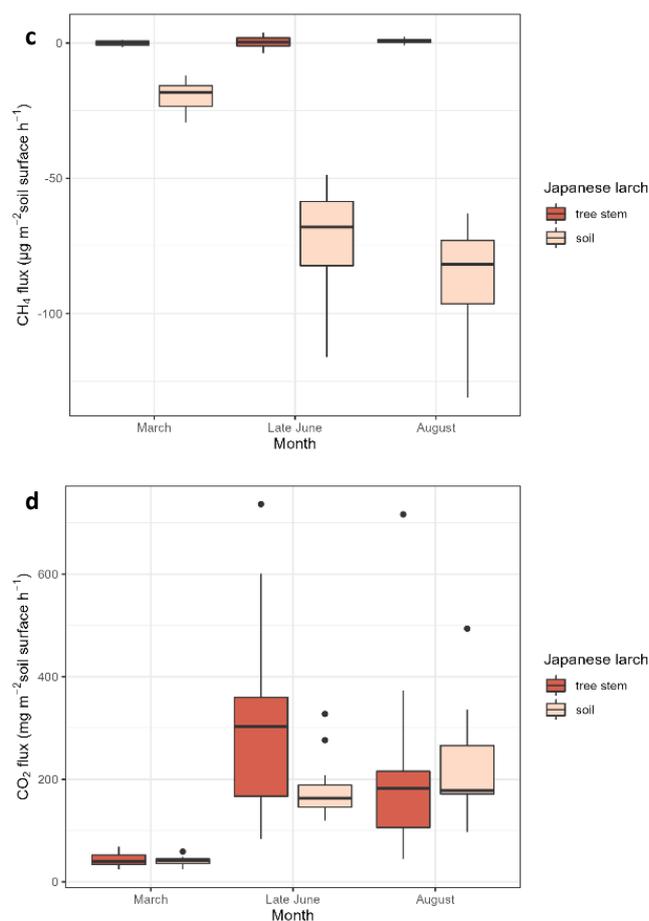


Figure 3. 8 Comparison of CH₄ (a, c) and CO₂ (b, d) fluxes between soil (light colour) and tree stem (dark colour) in English oak (blue) and Japanese larch (red) in 2020 (n=12 individual trees per species per measurement).

3.4.5 Daytime variation of tree stem gas flux

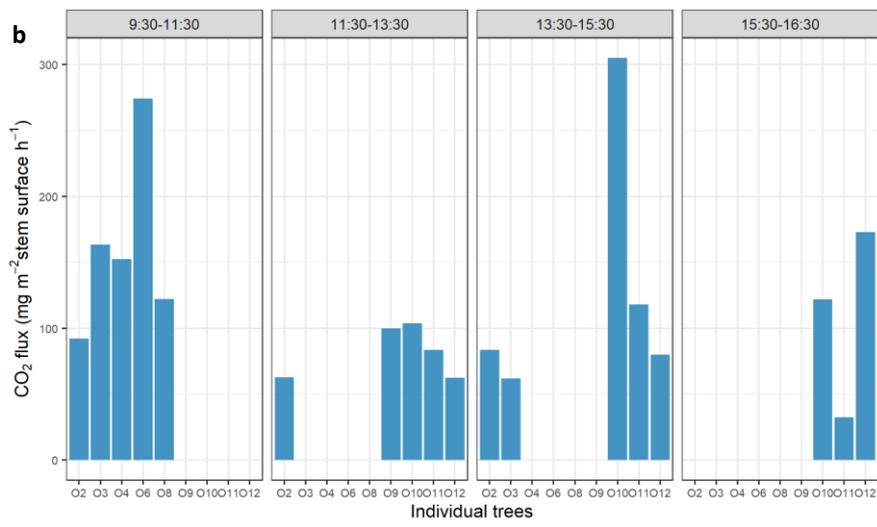
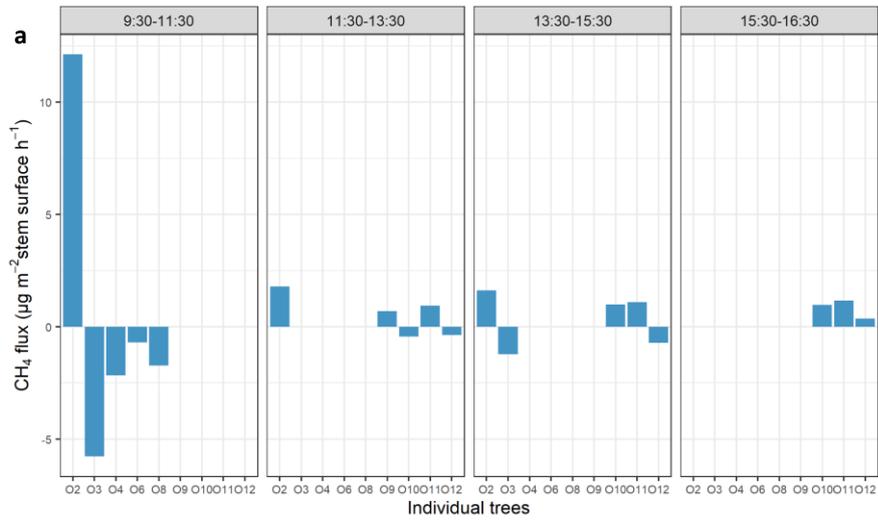
We measured tree stem CH₄ and CO₂ fluxes for both tree species between 9:30 to 16:30 of a day in early June (Table 3.5). Stem temperature of both tree species started to increase around 9 in the morning and reached the peak around 15:30. Therefore, we divided the daytime measurements into four time periods. For English oak trees, 80% of the trees (n=5) showed net CH₄ uptake from 9:30-11:30, whilst one tree showed large methane emission. Then 60% of the trees (n=5) showed net CH₄ release from 11:30-15:30, and from 15:30-16:30 all showed net CH₄ release again, though the latter was based on three trees only. For Japanese larch trees, 80% of the trees (n=5) showed net CH₄ uptake from 9:30-11:30, then 71% of the trees (n=7) showed net CH₄ release from 11:30-13:30, and 80% of the trees (n=10) showed net CH₄ uptake from 13:30-15:30, and although only two CH₄ flux from two trees from 15:30-16:30, they all showed net CH₄ release again. Tree stem CO₂ flux was comparable for both tree species during 9:30-11:30, but Japanese larch trees showed 2.6-3.6 times larger

stem CO₂ flux than English oak trees during 11:30-16:30. The daytime variation of English oak tree stem CO₂ flux was not as large as Japanese larch trees, which showed smaller stem CO₂ flux from 9.30-11.30 than during the rest of the day.

Due to leakage issues, we were not always able to include tree stem CH₄ and CO₂ fluxes from the same trees at each time interval (Fig. 3.9). However, we observed the same English oak trees (n=2) and Japanese larch trees (n=1) that were measured three times during 9:30-16:30 showing high variations in tree stem CH₄ flux, which switched from stem CH₄ uptake during 9:30-11:30 to stem CH₄ emission during 15:30-16:30. And the same English oak trees (n=2) and Japanese larch trees (n=2) were found constantly showing either stem CH₄ uptake or CH₄ emissions during 9:30-16:30, suggesting a large variation in intra-species of tree stem CH₄ flux.

Table 3. 5 The one-day measurements of tree stem CH₄ and CO₂ fluxes of both English oak and Japanese larch between 9:30-16:30 in early June 2020.

Tree species	Tree stem fluxes mean ± SD	Time interval			
		9:30-11:30	11:30-13:30	13:30-15:30	15:30-16:30
English oak	CH ₄ flux (µg m ⁻² stem surface h ⁻¹)	0.35 ± 6.86	0.52 ± 0.94	0.35 ± 1.24	0.84 ± 0.42
	CO ₂ flux (mg m ⁻² stem surface h ⁻¹)	160.96 ± 69.13	82.44 ± 19.61	129.70 ± 100.12	136.52 ± 79.82
Japanese larch	CH ₄ flux (µg m ⁻² stem surface h ⁻¹)	-1.78 ± 2.13	0.75 ± 0.93	-0.33 ± 0.79	0.92 ± 0.64
	CO ₂ flux (mg m ⁻² stem surface h ⁻¹)	139.54 ± 80.63	300.87 ± 83.49	334.40 ± 104.48	365.42 ± 185.42



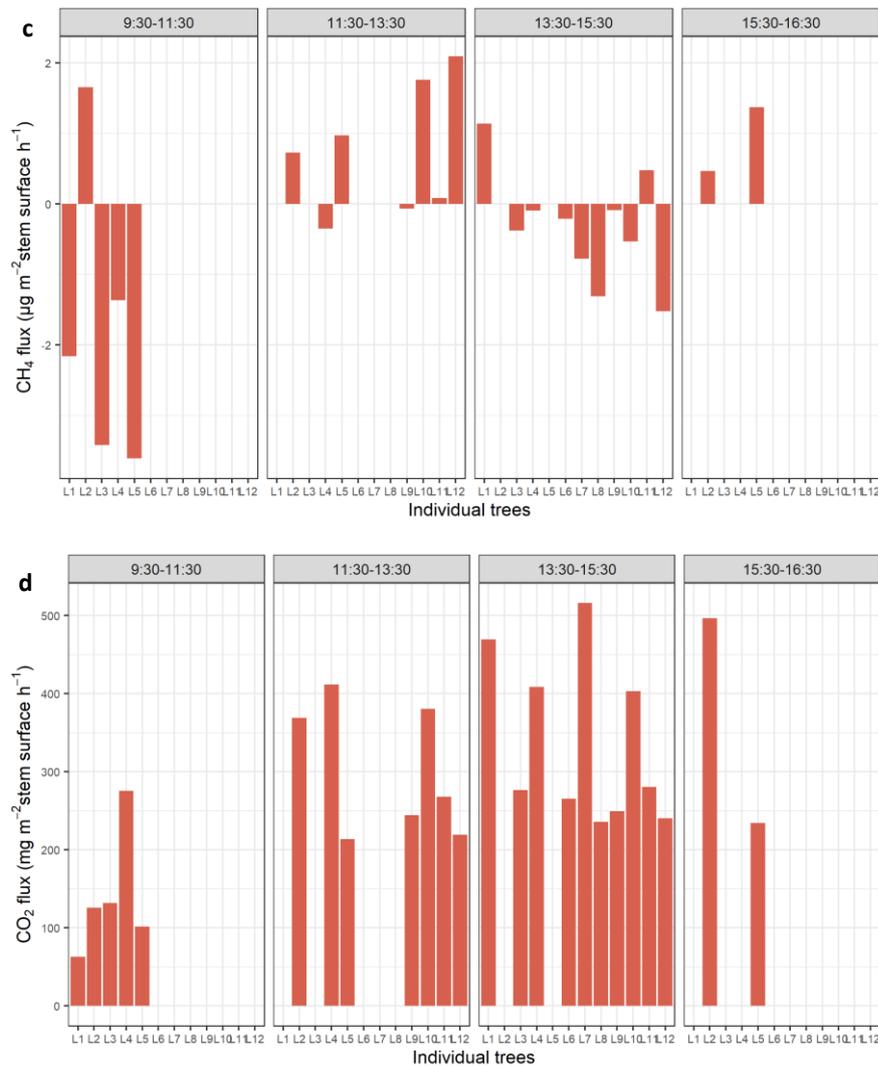


Figure 3. 9 Repeated measurements of English oak (blue) and Japanese larch (red) tree stem CH₄ (a, c) and CO₂ (b, d) fluxes by four different periods in early June 2020 (n=12 individual trees per tree species).

3.5 Discussion

3.5.1 Tree species effect on tree stem CH₄ and CO₂ fluxes & potential drivers

The results of English oak (*Quercus robur*) tree stem CH₄ flux in our study were comparable to other various deciduous tree species in a temperate upland forest with mean tree stem CH₄ flux $6.3 \pm 12 \mu\text{g m}^{-2} \text{ stem surface h}^{-1}$ (Warner et al, 2017). But Japanese larch (*Larix kaempferi*) tree stem CH₄ flux in our study was three orders of magnitude larger than Scots pine (*Pinus sylvestris* L.) with median tree stem CH₄ flux $0.005 \mu\text{g m}^{-2} \text{ stem surface h}^{-1}$ in a boreal forest (Machacova et al., 2016). Surprisingly, English oak and Japanese larch stems showed both stem CH₄ emission and uptake at our research site. However, other studies only reported tree stem CH₄ emissions from various oak tree species (*Quercus spp.*; *Quercus velutina*; *Quercus*

michauxii) and undetectable stem CH₄ flux from another larch tree species (*Larix gmelinii* (Ruprecht) Kuzeneva) during growing seasons in temperate upland forests, whilst soils acted as a net CH₄ sink (Wang et al., 2016; Warner et al., 2017; Pitz and Megonigal, 2017; Pitz et al., 2018). Although many studies have focused on tree stem CH₄ emissions in recent years, few studies have reported tree stem CH₄ uptake (Barba et al., 2019; Welch et al., 2019; Moldaschl et al., 2021; Barba et al., 2021). Sundqvist et al. (2012) have reported *in situ* CH₄ uptake by branches of four different tree species (*Picea abies*, *Betula pubescens*, *Sorbus aucuparia* and *Pinus sylvestris*) in a boreal forest. A recent study also observed both CH₄ emission and consumption by Norway spruce (*Picea abies*) shoot under field flux measurements in boreal forests, and novel metagenomic tools detected CH₄-producing methanogens and novel CH₄ oxidising monooxygenases, which gave further evidence of the net tree stem CH₄ exchange involving both CH₄ emission and uptake (Putkinen et al., 2021). Further studies are needed to understand net tree stem CH₄ exchange and its underlying mechanism in temperate forests.

Although our two study tree species - English oak and Japanese larch have different anatomy (vessels vs tracheid; ring-porous vs non-porous), which may affect gas diffusion and thus stem CH₄ flux (Barba et al., 2019), our results indicated there was no significant difference of tree stem CH₄ flux between English oak and Japanese larch trees during spring and summer of 2020. Similar results were reported in a temperate upland forest, where seven broadleaved tree species including both ring-porous and diffuse-porous did not show significantly different tree stem CH₄ flux during a growing season (May to October)(Pitz and Megonigal, 2017). In contrast, other studies reported that stem CH₄ emissions differed among tree species in temperate forests, which may be explained by the wood anatomy (e.g. wood density, lenticel density and sap flow rates) or disease resistance (e.g. susceptibility to fungal-mediated heart rot) (Covey et al., 2012; Wang et al., 2016, 2017; Warner et al., 2017; Pitz et al., 2018). However, it was suggested that the physiological and anatomical causes of tree stem CH₄ flux variations should be dependent on the source of tree stem CH₄ flux, whether from the soil or from the tree stem itself (Covey and Megonigal, 2019). In wetland and floodplain forests, temperate tree species with aerenchyma tissue can transport CH₄ produced in anoxic saturated soils or dissolved in groundwater (Terazawa et al., 2007, 2015; Gauci et al., 2010; Pangala et al., 2014, 2015; Sakabe et al., 2021). In contrast with those tree species that are adapted to flooding in wetland soils, most tree species in upland forests do not develop an

aerenchyma system (Machacova et al., 2013). We did not find a significant correlation between English oak and Japanese larch tree stem CH₄ flux and soil CH₄ flux at our research sites, and soils were a net CH₄ sink at all times. The origin of tree stem CH₄ production in temperate upland forests is most likely to be inside the tree stem itself, and high CH₄ concentrations have been observed in the heartwood of *Populus davidiana* and *Carya cordiformis* at 100-130 cm height and low CH₄ concentrations from the soil profile (Wang et al., 2016, 2017; Barba et al., 2021). Therefore, the variation in heartwood water content and wood density among tree species may regulate tree stem CH₄ production and gas diffusivity (Wang et al., 2017).

In addition, measuring stem CH₄ fluxes at different stem heights can be used as an indicator for the origin of stem CH₄ emission. It is hard for us to judge the effect of stem height on stem CH₄ and CO₂ flux due to the limited size of measurements. However, we did not find an effect of stem height on stem CH₄ flux of both tree species in August. Wang et al. (2016) also did not observe a significant effect of stem height on stem CH₄ flux in growing seasons, but tree stem CH₄ flux decreased with increasing height in a temperate upland forest (Wang et al., 2016). Most studies reported tree stem CH₄ emissions in both wetland and upland forests declined with increasing stem height, suggesting tree stem CH₄ source from deep layers of anaerobic soils (Terazawa et al., 2007; Pangala et al., 2013, 2014, 2017; Wang et al., 2016; Pitz and Megonigal, 2017; Barba, Poyatos and Vargas, 2019; Jeffrey et al., 2019). However, a recent study in a temperate upland forest found that the effect of tree stem height on stem CH₄ flux was only detected when other environmental variables were excluded in linear mixed models (Barba et al., 2021). In addition, Moldaschl et al. (2021) found the opposite effect of stem height on tree stem CH₄ emissions in two tree species (*Fraxinus excelsior* and *Populus alba*). It was expected that larger diameter trees can enhance tree stem CH₄ emissions due to a higher ability to transport soil produced CH₄ with a deep root system or higher internal CH₄ production with a larger heartwood diameter (Wang et al., 2017; Pitz et al., 2018; Barba et al., 2021). Except for the effect of stem height, we also did not observe a significant correlation between oak and larch tree stem CH₄ flux and DBH at our research sites, which may be due to the smaller diameter variance among individual tree stems. Considering the results of our study with both stem CH₄ uptake and emission, further studies are required to understand the pathway of tree stem CH₄ exchange in upland forests.

The results of tree stem CO₂ flux from English oak and Japanese larch at our research sites were comparable to other tree species in a temperate upland forest with mean tree stem CO₂ flux 301.02 ± 61.79 mg m⁻² stem surface h⁻¹ during the growing season measurements (Warner et al, 2017). Compared to English oak trees, we observed a significantly larger tree stem CO₂ flux of Japanese larch trees in March and June with 50.46 ± 12.52 and 361.24 ± 173.05 mg m⁻² stem surface h⁻¹, respectively. The variation of CO₂ concentrations inside tree stems among angiosperms and gymnosperms was listed by Teskey et al. (2008). It was reported that the origin of most tree stem CO₂ flux is from the respiring cells in the stems and roots, and the live cells of the inner bark and xylem in stems replied on tree species (Teskey et al., 2008), which may cause the difference in tree stem CO₂ flux rates. The significant difference in tree stem CO₂ flux between oak and larch trees we found at our research sites might be explained by the difference in respiring cells in the stems or a phenological difference.

In addition to the respiring cells of roots and stems, it was suggested that tree stem CO₂ flux can also originate from the rhizosphere (Teskey et al., 2008). However, we did not observe the significant effect of stem height on tree stem CO₂ flux in either tree species. Contrasting results have been reported showing tree stem CO₂ flux decreased with increasing height (Barba, Poyatos and Vargas, 2019). Although both oak and larch tree stem CO₂ flux at 45 cm height exhibited a positive correlation with soil CO₂ flux, the lack of stemheight effect on stem CO₂ flux suggested soil respiration may not be the origin of stem CO₂ emission.

3.5.2 Seasonal and diurnal variation on tree stem CH₄ and CO₂ fluxes & potential drivers

We did not observe seasonal variation in tree stem CH₄ flux over the spring and summer period of 2020, even though the soil was a constant net CH₄ sink, which exhibited a seasonal pattern with a significant increase during the growing season. Other studies showed similar results that tree stem CH₄ flux did not show a seasonal trend in temperate upland forests (Warner et al., 2017; Pitz and Megonigal, 2017; Pitz et al., 2018; Moldaschl et al., 2021). Researchers suggested that compared to high temporal frequency measurements (automated chamber, hourly frequency), manual measurements (manual chamber, monthly frequency) can fail to capture the large temporal variability of tree stem CH₄ flux (Barba et al., 2019), which might explain the lack of a seasonal pattern of tree stem CH₄ flux in our study. Measurements from a single hickory tree (*Carya cordiformis*) in a temperate upland forest

showed that seasonal variations in tree stem CH₄ emission could be detected by using high-frequency measurements (Barba, Poyatos and Vargas, 2019), which was confirmed by a later study. Combining manual and automated flux measurements of 18 hickory trees in a temperate upland forest, only automated measurements exhibited a seasonal trend in tree stem CH₄ flux which peaked at the end of summer and started decreasing around the end of autumn, while manual measurements of more trees were better at addressing the integration of intraspecific variation (Barba et al., 2021). We also observed large intraspecific variations in tree stem CH₄ flux of both English oak and Japanese larch trees during daytime measurements at our study sites (see discussion below).

We did not observe a relationship between tree stem CH₄ flux and soil moisture, soil temperature or air temperature during the entire measuring period. Similar results were reported in other studies in temperate upland forests (Pitz and Megonigal, 2017; Warner et al., 2017). In contrast, it was indicated that in temperate upland forests, tree stem CH₄ emission increased with higher soil moisture (Maier et al., 2018; Barba, Poyatos and Vargas, 2019; Barba et al., 2021; Moldaschl et al., 2021), which may be due to enhanced soil CH₄ production in deeper soil layers and/or increased stem respiration and transpiration stream. Canopy openness (the percentage of the sky unobscured by the forest canopy) is one of the indicators of forest structure (Russavage et al., 2021). Open canopy with more sunlight through the forest floor can lead to higher soil temperature and soil water content (Gray, Spies and Easter, 2002; Cai et al., 2021) which might alter CH₄ emissions. Although our two sites differed in canopy openness when measured at the end of the experiment in September, we did not measure canopy openness monthly during the entire study period and were unable to explore any correlation between canopy openness and tree stem CH₄ flux.

However, both tree species presented a seasonal pattern on tree stem CO₂ flux with larger stem CO₂ flux in summer than in spring. Similar results were also reported in temperate upland forests, in which tree stem CO₂ flux increased over a growing season, explained by soil or air temperature and soil water content (Warner et al., 2017; Pitz et al., 2018; Barba, Poyatos and Vargas, 2019; Barba et al., 2021). The results were also confirmed in our study which we found both English oak and Japanese larch tree stem CO₂ flux was positively correlated to soil temperature and air temperature and negatively correlated to soil moisture.

Due to the time limitation, we did not measure the tree stem CH₄ and CO₂ flux during both daytime and night-time, but a large daytime variation of tree stem CH₄ flux of both tree species was observed in our study. We found most oak and larch trees showed stem CH₄ uptake during the early morning (9:30-11:30), and large intraspecific variations in tree stem CH₄ flux of both trees species during the afternoon (11:30-15:30), then this pattern shifted with all measured oak and larch tree stem CH₄ emissions during the late afternoon (15:30-16:30). Similar results were also reported that automated measurement of *Liriodendron tulipifera* and *Carya cordiformis* presented a diurnal pattern of tree stem CH₄ flux which may be explained by sap flow and stem temperature changes (Pitz and Megonigal, 2017; Barba, Poyatos and Vargas, 2019). It was suggested that *in situ* high-frequency measurements of diurnal variations can potentially reveal the pathway of tree stem CH₄ emissions, indicating CH₄ transporting from the soil via transpiration-driven mass flow or pressurized ventilation, rather than from heartwood by diffusivity across the stem (Covey and Megonigal, 2019). However, it is still hard to fully understand the underlying mechanism of tree stem CH₄ source, as we found oak and larch tree stem CH₄ flux switch between net CH₄ consumption and net CH₄ emission during the daytime. We observed Japanese larch trees stem CO₂ flux increased over daytime measurement, while the daytime variation of English oak tree stem CO₂ flux was not evident. Barba et al. (2019) reported that the diurnal variation of stem CO₂ flux was associated with stem temperature and sap flow changes. However, other factors such as precipitation, the water status of the living cells and the gas diffusion pathway between the stem and the atmosphere, in addition to cellular respiration can also affect tree stem CO₂ flux (Teskey et al., 2008, 2017). Barba et al. (2019) have suggested that understanding the relationship between tree stem CO₂ and CH₄ flux can help to model and estimate stem CH₄ flux as the stem CO₂ flux mechanism has been more widely studied (Teskey et al., 2008, 2017). We did not observe a significant relationship between tree stem CH₄ flux and stem CO₂ flux over the entire study period at our research sites, although it has to be acknowledged that the data set was limited to four measurement occasions. In contrast, a positive correlation between stem CH₄ and CO₂ fluxes was reported for a more detailed data set over a growing season in a temperate upland forest (Barba et al., 2021). This correlation was attributed to gas diffusivity heterogeneity through the wood, which might similarly affect both gases (Barba et al., 2019). However, the large variations in tree stem CH₄ flux make it complicated to model tree stem CH₄ flux based on stem CO₂ flux (Barba et al., 2021).

3.5.3 The role of tree stem and soil CH₄ fluxes in forests on mineral soils

We up-scaled tree stem CH₄ flux at 45 cm stem height to the site level assuming homogenous flux for the entire height of the stems over the spring and summer period of 2020, but spatial variability and temporal variability need to be addressed for up-scaling to the site or forest scale in the future. At our research sites, English oak and Japanese larch tree stem CH₄ emissions can offset soil CH₄ uptake ranging from 0.4% to 1.6% during a growing season (March-August). The results were close to other studies, showing a conservative estimation of 1% offset soil CH₄ sink by tree stem CH₄ emissions in upland deciduous temperate forests (Pitz and Megonigal, 2017; Moldaschl et al., 2021) and 0.8% offset soil CH₄ uptake by shoot CH₄ emissions in a dry *Pinus sylvestris* site (Machacova et al., 2016). However, those estimates were far lower than that reported in another measurement from a riparian cottonwood forest, which indicated that the tree stem CH₄ emission offset 86% of the estimated soil CH₄ uptake (Flanagan et al., 2021). Tree stem CH₄ flux has not been included in the global CH₄ budget yet (Saunois et al. 2020) and it has been estimated that tree stem CH₄ emissions might contribute less than 0.4% to the global total natural and anthropogenic CH₄ sources (Wang et al., 2021). However, it is still very challenging to up-scale and model tree stem CH₄ dynamics on a global scale, because of the variance of stem CH₄ exchange in different local and regional upland forests (Pitz and Megonigal, 2017; Wang et al., 2021) and limited knowledge of the underlying mechanism of tree stem CH₄ production, consumption and transportation (Barba et al., 2019). In addition, the CH₄ exchange from shoots and branches (Sundqvist et al., 2012; Machacova et al., 2016; Putkinen et al., 2021) should also be included in the estimation of tree CH₄ flux contribution in a global budget. New strategies of up-scaling stem CH₄ flux have been studied by using three-dimensional (3D) photogrammetry to estimate tree stem surface area (Jeffrey et al., 2020) and considering the variation of tree stem CH₄ flux at different stem heights (Jeffrey et al., 2019; Moldaschl et al., 2021). To address the challenge of spatial variability and temporal variability in up-scaling tree stem CH₄ flux to a site level, high-frequency measurements (automated chamber, hourly frequency) of tree stem CH₄ flux were taken at three stem heights in a temperate upland forest in the next chapter.

3.6 Conclusion

In this study, we measured tree stem and soil CH₄ and CO₂ fluxes from English oak and Japanese larch during the spring and summer period of 2020 in a temperate upland forest. All

sampled oak and larch trees showed both stem CH₄ emissions and uptake over the entire studying period, while soils only acted as a net CH₄ sink at two research sites. No significant differences in tree stem CH₄ flux between the two tree species were found during the growing season, but larch trees showed significantly larger stem CO₂ flux than oak trees in March and June. Tree stem CH₄ flux of English oak and Japanese larch did not follow a seasonal pattern, but tree stem CO₂ flux exhibited seasonal variations, which were correlated with soil moisture, soil temperature and air temperature. Although tree stem CH₄ and CO₂ fluxes at 45 cm and 130 cm above the soil were only determined once, we did not observe any effect of stem height on stem CH₄ and CO₂ fluxes of both tree species. Compared to tree stem CO₂ flux, large daytime and intra-specific variations in tree stem CH₄ flux of both tree species were observed. We tried to identify potential drivers of tree stem CH₄ flux in our study, in order to understand the underlying mechanism and pathway of tree stem CH₄ exchange in temperate upland forests. However, the large variations in tree stem CH₄ flux including both CH₄ emissions and uptake, made it complicated to model and up-scale tree stem CH₄ flux to a site level. Further studies are recommended to use high-frequency measurements of tree stem CH₄ and CO₂ fluxes to address the spatial and temporal variability.

4. Diurnal Patterns of White Poplar (*Populus alba*) Tree Stem CH₄ and CO₂ Fluxes Using High-frequency Measurements in a Temperate Woodland

4.1 Abstract

In recent years, novel studies about tree stem CH₄ emissions have drawn worldwide attention. High temporal variability in stem CH₄ fluxes hampers the accurate estimation of these fluxes in forests, but this can be addressed by using high-frequency measurements. However, few studies have reported high-frequency measurements of tree stem CH₄ flux by using automated chambers in temperate trees to date. In order to identify the underlying mechanism of tree stem CH₄ exchange, high-frequency measurements (1.5 hourly frequency, n>14,000) of three white poplar (*Populus alba*) tree stem CH₄ and CO₂ fluxes were taken at heights of 45, 130 and 200 cm above the soil from mid-June to mid-August (60 days) in a temperate woodland. We observed a large hour-to-hour variation in stem CH₄ flux with both CH₄ uptake and emissions occurring. The average frequency of stem net CH₄ uptake on a daily basis was 50.7% ± 1.8. There was no height effect on tree stem CH₄ flux (P=0.657), but CO₂ flux was significantly larger at 130 cm height than that at 45 cm height only during the early evening (16:00-20:00) (P<0.01). While there was no marked diurnal pattern, we found stem CH₄ flux at all three heights was significantly larger after midnight (00:00-04:00) than during dawn and afternoon (04:00-16:00) periods, and compared to the evening (20:00-24:00), stem CH₄ flux was significantly smaller during the morning (08:00-12:00) (P<0.05). All three of the poplar tree stem CO₂ flux exhibited a clear diurnal pattern, with larger CO₂ flux during the night-time (20:00-04:00) than the rest of the day, and smallest CO₂ flux during the morning (08:00-12:00). We did not find any significant correlations between stem CH₄ flux and soil CH₄ flux, soil moisture and initial CH₄ concentration at three different heights, and soils mostly exhibited a net CH₄ sink. The results we observed suggest that biologically *in situ* tree stem produced CH₄ was the major source of stem CH₄ emission in these trees. However, the large hour-to-hour variation in stem CH₄ flux with a large proportion of stem CH₄ uptake makes it complicated to understand the pathway and mechanism of stem CH₄ flux. We believe that the source of stem CO₂ flux is from the respiring cells from stems and roots, and this respiratory CO₂ can dissolve and be transported via transpiration stream and sap flow.

4.2 Introduction

In recent years, novel studies on tree stem CH₄ emission in upland forests have drawn attention on a global scale (Wang et al., 2016, 2017; Machacova et al., 2016; Warner et al., 2017; Pitz and Megonigal, 2017; Maier et al., 2018; Pitz et al., 2018; Barba, Poyatos and Vargas, 2019; Moldaschl et al., 2021; Barba et al., 2021). A large proportion of studies have found that in floodplain and wetland forests, CH₄ is biologically produced in anoxic saturated soils or dissolved in groundwater, and then absorbed by roots and transported in stems through intercellular spaces and aerenchyma tissue via the transpiration stream, and finally diffused by tree stem to the atmosphere (Terazawa et al., 2007, 2015; Rice et al., 2010; Gauci et al., 2010; Pangala et al., 2013, 2015, 2017; Jeffrey et al., 2019; Jeffrey et al., 2021b; Sakabe et al., 2021). Compared to wetland soils, in upland (well-drained) temperate forests on mineral soils where soil is a net CH₄ sink, it was believed that tree stem CH₄ was produced biologically *in situ* inside the heartwood (Covey et al., 2012; Wang et al., 2016, 2017; Yip et al., 2018; Barba et al., 2021). Most studies taking measurements of stem CH₄ fluxes monthly (or less frequent) showed large variations between individual trees and large temporal variations of fluxes within a given tree stem (Moldaschl et al., 2021; Flanagan et al., 2021; Chapter Three). To date, limited studies have carried out high-frequency (hourly frequency), within-day measurements of both stem CH₄ and CO₂ fluxes (Pitz and Megonigal, 2017; Barba, Poyatos and Vargas, 2019; Barba et al., 2021). However, the high-frequency measurements demonstrated a large variation in the magnitude of stem CH₄ flux (Barba, Poyatos and Vargas, 2019; Barba et al., 2021). We also observed large daytime (9:30-16:30) variations in tree stem CH₄ fluxes including both CH₄ emission and uptake of English oak (*Quercus robur*) and Japanese larch (*Larix kaempferi*) trees in a temperate upland forest (Chapter Three). It was suggested that high temporal frequency measurements (hourly frequency) using automated chambers are required to capture temporal variability and hotspots of stem CH₄ fluxes, whilst spatial variability (between tree stem heights of individual trees and between trees at the same stem height) of stem CH₄ fluxes can be addressed by using manual chambers (Barba, Poyatos and Vargas, 2019; Barba et al., 2019, 2021). More studies are required to determine stem CH₄ and CO₂ fluxes via high-frequency measurement on various tree species and environmental conditions (wet or dry soil conditions) in temperate upland forests.

However, the underlying mechanisms of tree stem CH₄ emissions remain poorly understood due to the large temporal and spatial variability in tree stem CH₄ emissions in upland forests (Covey and Megonigal, 2019; Barba et al., 2019, 2021). To identify the main mechanisms underpinning tree stem CH₄ emissions, studying diurnal variations can potentially reveal the pathway of tree stem CH₄ emissions. In temperate upland forests, diurnal patterns of stem CH₄ flux correlated positively with the transpiration rate suggesting that stem CH₄ production was biologically produced inside the soil and then transported via transpiration-driven mass flow or pressurized ventilation, rather than produced in the heartwood followed by radial diffusion across the stem (Barba et al., 2019; Covey and Megonigal, 2019). This was further confirmed by high-frequency measurements of a single *Carya cordiformis* and a single *Liriodendron tulipifera* tree stem CH₄ fluxes in temperate upland forests, showing a clear diurnal pattern of stem CH₄ flux, which was related to sap flow rates (Pitz and Megonigal, 2017; Barba, Poyatos and Vargas, 2019). However, in the study by (Pitz and Megonigal, 2017), the diurnal pattern of tree stem CH₄ flux was not consistent for two tree species at the same forest site measured over a 3-day period, which complicates understanding of tree stem CH₄ flux pathways.

Measuring stem CH₄ fluxes at different stem heights and in the soil can also help elucidate the underlying mechanisms of stem CH₄ flux. In both wetland and upland forests, studies reported that tree stem CH₄ emissions declined with increasing stem height, suggesting tree stem CH₄ production was originated from deep layers of anaerobic soil CH₄ production (Terazawa et al., 2007; Pangala et al., 2013, 2014, 2017; Wang et al., 2016; Pitz and Megonigal, 2017; Barba, Poyatos and Vargas, 2019; Jeffrey et al., 2019). Manual measurements of stem CH₄ flux can reach up to 2 m height of the tree stems using cheap, lightweight and flexible manual chambers (Siegenthaler et al., 2016; Jeffrey et al., 2020). However, automated chambers are more expensive, heavier and more difficult to be constructed and transported than manual chambers, which hinders high-frequency measurements of stem gas fluxes. Future studies are needed to study high-frequency measurements of stem CH₄ and CO₂ fluxes at various stem heights.

Compared to stem CH₄ flux, the mechanisms of CO₂ emissions from the stems to the atmosphere have been more widely studied and described (Teskey et al., 2008, 2017). The diurnal pattern of stem CO₂ flux varied with sap flow rates, temperature, sap pH, the water

status of the living cells, the gas diffusion pathway between the stem and the atmosphere, and CO₂ concentrations inside the xylem sap (McGuire and Teskey, 2002, 2004; Saveyn et al., 2008; Teskey et al., 2008, 2017). As stem CO₂ flux and stem CH₄ flux may share underlying mechanisms such as a similar pattern of axial diffusion across common physical barriers, simultaneous measurement of these stem fluxes may help to understand the main pathways of stem CH₄ flux (Barba et al., 2019; Megonigal, Brewer and Knee, 2020).

This study therefore aimed to 1) determine high-frequency (within-day) variation in stem CH₄ and CO₂ fluxes of three white poplar (*Populus alba*) trees over a 2-month summer period in a temperate, managed woodland on mineral soil in the UK, and 2) explore the relationship between soil CH₄ uptake or production with stem CH₄ emissions by comparing high-frequency CH₄ (and CO₂) fluxes at different tree stem heights and from the soil.

The hypotheses were:

1. Tree stem CH₄ flux exhibits large hour-to-hour variation during the whole period.
2. Tree stem CH₄ and CO₂ fluxes both show a consistent diurnal pattern, with larger fluxes during the daytime than during the night-time.
3. Tree stem CH₄ and CO₂ fluxes decrease with increasing stem height, because these fluxes are partially derived from the deeper layers of soil, whilst soils act as a net CH₄ sink.

4.3 Methods

4.3.1 Study site

The research was carried out in a managed, planted woodland on a mineral soil, located on the campus of the University of York, United Kingdom, at 53°56'38''N, 0°03'29''W (UK Grid Reference SE619501). The soil is a well-draining, sandy clay loam with a pH of 7.2. Mean annual maximum and minimum temperatures were 13.6 °C and 5.7 °C, respectively, and the annual total precipitation was 603.2 mm (1981 to 2010, data from Church Fenton station, located 17.6 km southwest of the forest (UK Met Office Library & Archive; www.metoffice.gov.uk)). The research plot was dominated by white poplar (*Populus alba*) scattered with a few horse chestnut (*Aesculus hippocastanum*) and alder (*Alnus glutinosa*). Three white poplar trees were selected for this study (Table 4.1).

Table 4. 1 The characteristics of selected white poplar trees (n=3 trees) in woodland at the campus of the University of York

Type of shedding leaves	Tree species	Common name	DBH ^a (cm) Mean ± SD	Tree height (m) Mean ± SD	Tree anatomy
Deciduous broadleaf	<i>Populus alba</i>	white poplar	35.3 ± 4.7	25.4 ± 1.1	diffuse- to semi-ring-porous

^aDBH: Diameter at breast height

4.3.2 Experimental design and chamber installation

To study the short-term (within-day) variation in white poplar stem CH₄ and CO₂ fluxes and their potential drivers, *in situ* high-frequency flux measurements from three trees were continuously measured (every 1.5 h) at 45, 130 and 200 cm above the soil surface, and from the soil near each tree (n=1 per tree) from 17th June until 15th August 2021 (60 days).

We installed a PVC collar (5 cm height, 20 cm inner diameter, 20.4 cm outer diameter) at each of three stem heights (n=9 collars), and sealant (Zero VOC sealant, Ecomerchant, UK) was applied to fill stem cracks and the gap between the collars and stems. To compare tree stem fluxes with soil fluxes, a PVC collar (10 cm height, 20 cm inner diameter, 20.4 cm outer diameter) was located 1-1.5 m from each tree stem (n=3 collars) and inserted into the litter/soil layer leaving about 5 cm collar above the soil surface. A 20-cm diameter opaque multiplexed automatic long-term chamber (LI-8100-101, Li-Cor, Lincoln, Nebraska, USA) was installed above each tree stem and soil collar, and every 1.5 h automatically each chamber closed for 6 min, forming an air-tight sealing around the outside of collars with a rubber gasket, to take a gas flux measurement (Fig. 4.1). Ratchet straps (5m × 25mm, Hila tools, CPC, UK) were used to fix the location of automated chambers on the tree stems. All PVC collars were installed in March 2021 and remained in place throughout the experimental period. The function of all Li-Cor chambers was checked regularly during the experimental period.



Figure 4. 1 Automatic long-term chambers (LI-8100-101, Li-Cor, Lincoln, Nebraska, USA) on soils and three white poplar stems at 45, 130 and 200 cm above the soil surface. Tree 1, tree 2 and tree 3 were marked shown as in the pictures.

4.3.3 Tree stem and soil flux measurements

Tree stem and soil CH₄ and CO₂ concentrations in the headspace of a closed chamber were measured at a rate of 1Hz using a Fast Greenhouse Gas Analyser (FGGA, Los Gatos Research, Inc., Mountain View, CA, USA) and a LI-8100 infra-red gas analyser (IRGA, Li-Cor, Lincoln, Nebraska, USA). The automatic long-term chambers were controlled by a multiplexer (Electronic workshop, Department of Biology, University of York). The multiplexer was connected to the IRGA and the FGGA (Fig. 4.2), and sampled each chamber sequentially such that chambers were measured once every 1.5 hours. During a gas flux measurement, a chamber was closed over a 6-min period and with 1 min in-between measurements. To determine the volume of the gas enclosed by the automatic chambers and experimental setup, distances from the stem or soil surface to the top of the collar were measured inside the collar. The volume of the Bev-A-Line Tubing (0.3 cm inner diameter, Cole-Parmer, UK) to connect the chamber and the gas analysers and multiplexer, and the inner volume of the gas analysers were also determined.

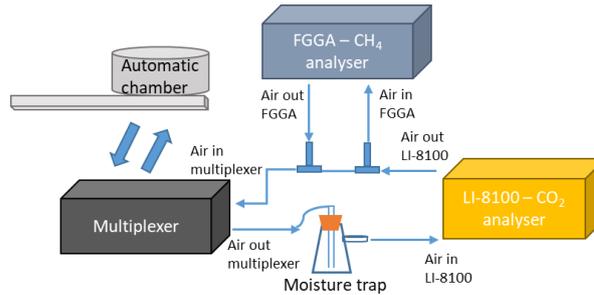


Figure 4. 2 Pictures of the experimental setup of tree stem and soil gas flux measurements.

4.3.4 Stem and soil gas flux calculations

Tree stem and soil CH₄ and CO₂ fluxes were calculated from the 1Hz concentration data collected by the FGGA and LI-8100 instruments using SoilFluxPro software (v4.2.1; Li-COR, Lincoln, Nebraska). Stem and soil CO₂ fluxes were obtained from both the FGGA and LI-8100. The CO₂ fluxes from both instruments showed a strong linear relationship ($y=0.8992+5.7928x$, $R^2=0.96$), but we used the CO₂ fluxes from the LI-8100 rather than from the FGGA as the airstream was measured first by the LI-8100 which therefore may provide slightly more accurate CO₂ flux data.

Gas flux was calculated using the following equation:

$$F = \left(\frac{dC}{dt}\right) \times \frac{PV}{ART}$$

where F is the flux of the particular gas, dC/dt is the change in concentration over time (ppm s⁻¹), P is atmospheric pressure, T is Kelvin temperature, R is the universal gas constant, A is the chamber surface area and V is the system volume. Flux units are reported in μg m⁻² h⁻¹ or mg m⁻² h⁻¹.

We calculated tree stem and soil CH₄ and CO₂ fluxes using linear regression of the concentration measurements obtained during each 6-min chamber closure. The first 40 s (tree stem and soil fluxes) were discarded when the air in the system was mixing immediately after chamber closure. To obtain a better fit of the linear regression, each linear regression of tree stem and soil CH₄ flux used the window length from 40-300 s, and each linear regression of tree stem and soil CO₂ flux used the window length from 40-160 s.

For quality control of gas fluxes, we firstly checked for leakage issues during each flux measurement and both CH₄ and CO₂ fluxes were removed when continuous fluctuation of the

CO₂ concentration was observed during chamber closure. We removed 18.2% of the tree stem CO₂ flux measurements (1295 out of a total of 7124) and 18.3% of the tree stem CH₄ flux measurements (1303 out of a total of 7124) due to leakage or other data quality issues. We removed 13.1% of the soil CO₂ flux measurements (311 out of a total of 2372) and 13.5% of the soil CH₄ flux measurements (320 out of a total of 2372). Then we checked if both CH₄ and CO₂ flux measurements were above the minimum detectable flux (MDF) (Courtois et al., 2019), which removed a further 39 tree stem CH₄ measurements (0.55% of the data) and 2 soil CH₄ flux measurements (0.08% of the data) that did not meet the MDF standard. All other valid fluxes were kept regardless of R² of CH₄ and CO₂, because small flux rates tended to show lower R². All tree stem and soil CH₄ and CO₂ fluxes data were lost due to a power cut from 15:00 on 13th July to 17:00 on 14th July and from 18:00 on 4th August to 14:00 on 9th August 2021. No tree stem and soil CH₄ fluxes data were included due to the FGGA equipment failure from 15:00 on 21st June to 14:30 on 24th June and from 14:00 on 29th July to 15:00 on 2nd August 2021.

To detect any overall diurnal pattern of tree stem and soil CH₄ and CO₂ fluxes throughout the experiment, daily CH₄ and CO₂ fluxes of each tree stem were first normalised using the method of min-max normalization, which removed the magnitude differences of CH₄ and CO₂ fluxes between dates and allowed the observation of a diurnal pattern (Wu et al., 2021). Normalised stem and soil CO₂ flux values ranged between 0 and 1, and CH₄ flux values ranged between -1 and 1.

4.3.5 Environmental measurements

One data logger (GP1, Delta-T Devices, Cambridge, UK) was installed to measure air temperature (10 cm above the soil, in the shade), volumetric soil moisture content (0-6 cm depth) and soil temperature (10 cm depth) continuously every four hours throughout the experiment (from 16:00 on 24th June 2021 onwards). Volumetric soil moisture was measured with ML2x theta probes (Delta-T Devices Ltd.), and soil and air temperature were measured with ST1 probes (Thermistor type 2K, Delta-T Devices Ltd.). The diameter (at 45, 130 and 200 cm above the soil) and height of each tree were measured using a measuring tape and a clinometer. To observe the overall diurnal pattern of soil moisture, air temperature, soil temperature and initial atmospheric CH₄ at the start of each flux measurement throughout the experiment (as an indicator of *in situ* atmospheric concentrations for non-biased CH₄ flux

calculation), daily environmental variables were first normalised to values ranging between 0 and 1 using the method of min-max normalization. White poplar has black diamond-shaped lenticels on the stems. Stem lenticel density within each PVC collar (400 cm²) was estimated using 2 × 2 cm grids by ImageJ (National Institutes of Health, Bethesda, Maryland, USA) (Pangala et al., 2014).

4.3.6 Statistical analysis

All the statistical tests were performed in SPSS Statistics Software (Version 28; IBM Corp.). To further visualise the diurnal variation of tree stem and soil gas flux and environmental variables, normalised data were first binned into four-hourly bins, which were 02:00 (00:00 – 03:59), 06:00 (04:00 – 07:59), 10:00 (08:00 – 11:59), 14:00 (12:00 – 15:59), 18:00 (16:00 – 19:59), and 22:00 (20:00 – 23:59), and mean value of normalised tree stem and soil CH₄ and CO₂, soil moisture, air temperature, soil temperature and initial atmospheric CH₄ concentration were calculated and used for statistical analysis. The gas flux and environmental data met the assumptions of homogeneity of variance (Levene's test) and normality (Kolmogorov-Smirnov test). Visual inspection of residual plots and normality test (Kolmogorov-Smirnov test) of the residuals did not reveal any obvious deviations from homoscedasticity or normality. We performed linear mixed effects analysis to assess the diurnal variation in normalised tree stem and soil CH₄ and CO₂ fluxes and initial atmospheric CH₄ over time. For tree stem CH₄ and CO₂ fluxes and initial atmospheric CH₄ concentration, the fixed factors time of day and tree height (and their interactions) and the random factor individual tree were included in the model. For soil CH₄ and CO₂ fluxes, the fixed factors time of day and the random factor individual soil collar were included in the model. Due to the observed significant time of day × tree height interaction for tree stem CO₂ flux, and in order to check the diurnal pattern on normalised soil temperature, air temperature and soil moisture, a Kruskal-Wallis test was conducted on tree stem CO₂ flux, soil temperature, air temperature and soil moisture at each 4-hourly bin. Posthoc tests (Mann-Whitney U test with Bonferroni correction) were applied if a significant effect (P<0.05) was found. Correlations between normalised tree stem and soil CH₄ and CO₂ fluxes, and soil moisture, soil temperature, air temperature and initial atmospheric CH₄ concentration were analysed using the Spearman's rank method.

4.4 Results

4.4.1 Daily weather conditions

We did not observe a high daily variation (4-hourly frequency) in air temperature, soil temperature and volumetric soil moisture content during the entire study (Fig. 4.3). Mean air and soil temperature (at 10 cm depth) during the entire study were 16.14 ± 3.60 (mean \pm SD) and 16.02 ± 1.36 °C, ranging from 8.9 to 31.3, and 13.2 to 20 °C, respectively, and the volumetric soil moisture content (0-6 cm depth) ranged from 0.13 to 0.43 $\text{m}^3 \text{m}^{-3}$ with a mean of $0.24 \pm 0.08 \text{m}^3 \text{m}^{-3}$.

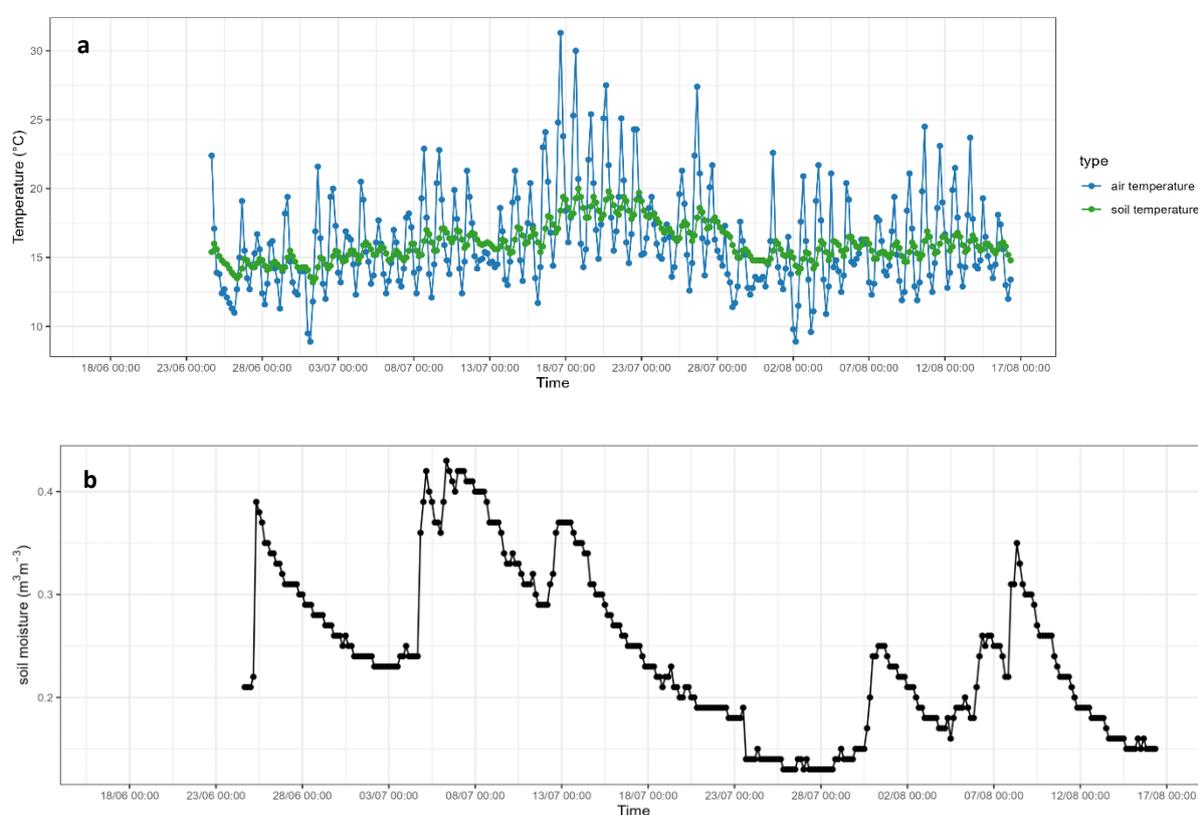


Figure 4. 3 Data of air (blue) and soil (green) temperature (a) and volumetric soil moisture content (b) during 24th June to 16th August 2021 (54 day).

4.4.2 High-frequency measurement of CH₄ and CO₂ fluxes

The stem CH₄ flux of the poplar trees at 45, 130 and 200 cm above the soil showed a large variation during the 2-month measurement period with both net stem CH₄ uptake and emission, varying from -121.10 to 196.78, -148.68 to 56.85, -104.43 to 147.37 $\mu\text{g m}^{-2}$ stem surface h^{-1} , respectively (Fig. 4.4a, b, c). The average frequency of stem net CH₄ uptake on a daily basis was 51.31%, 52.12% and 48.72% within tree 1, tree 2 and tree 3, respectively.

Mean stem CH₄ flux of the three poplar trees at 45, 130 and 200 cm above the soil were 0.66 ± 21.63 , 0.29 ± 20.95 and 0.93 ± 19.57 $\mu\text{g m}^{-2}$ stem surface h^{-1} , respectively. Compared to stem CH₄ flux, stem CO₂ flux showed less variation at 45, 130 and 200 cm above the soil with 98.13 ± 50.57 , 91.76 ± 47.06 and 72.55 ± 40.27 mg m^{-2} stem surface h^{-1} , respectively (Fig. 4.4d, e, f).

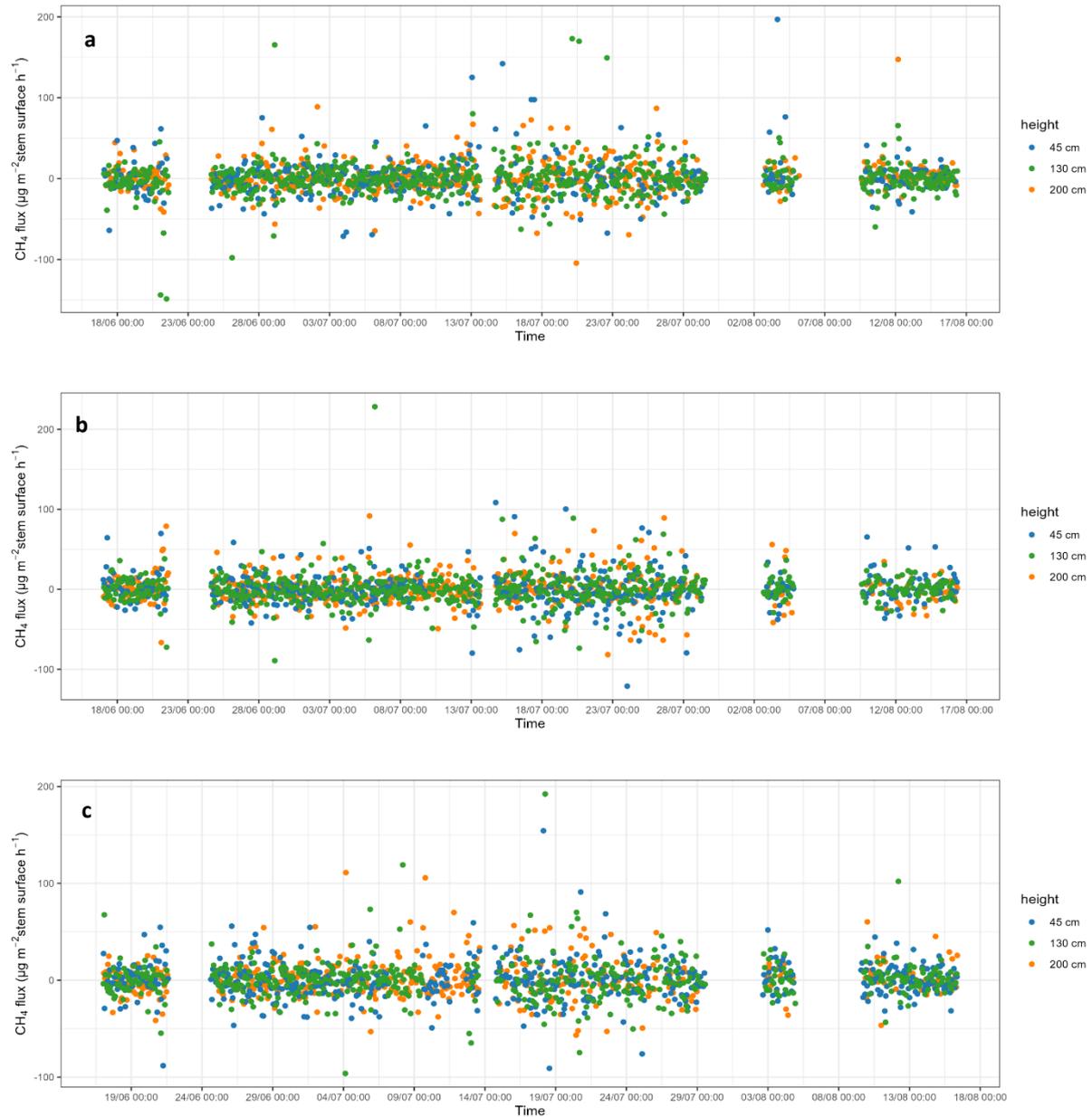




Figure 4.4 Daily data of three white poplar tree stem CH₄ and CO₂ fluxes (tree 1: a, d; tree 2: b, e; tree 3: c, f) at 45 (blue), 130 (green) and 200 cm (orange) height during 17th June to 16th August 2021 (60 days).

The high-frequency measurements of soil CH₄ flux showed that soil mostly exhibited a net CH₄ uptake on a daily basis and the average frequency of soil CH₄ uptake on a daily basis was $96.54\% \pm 0.02$ (Fig. 4.5a). During the measuring period, CH₄ and CO₂ fluxes of the mineral soil at the study site were $-40.86 \pm 24.58 \mu\text{g m}^{-2} \text{ soil surface h}^{-1}$ and $270.74 \pm 172.39 \text{ mg m}^{-2} \text{ soil surface h}^{-1}$, respectively (Fig. 4.5).

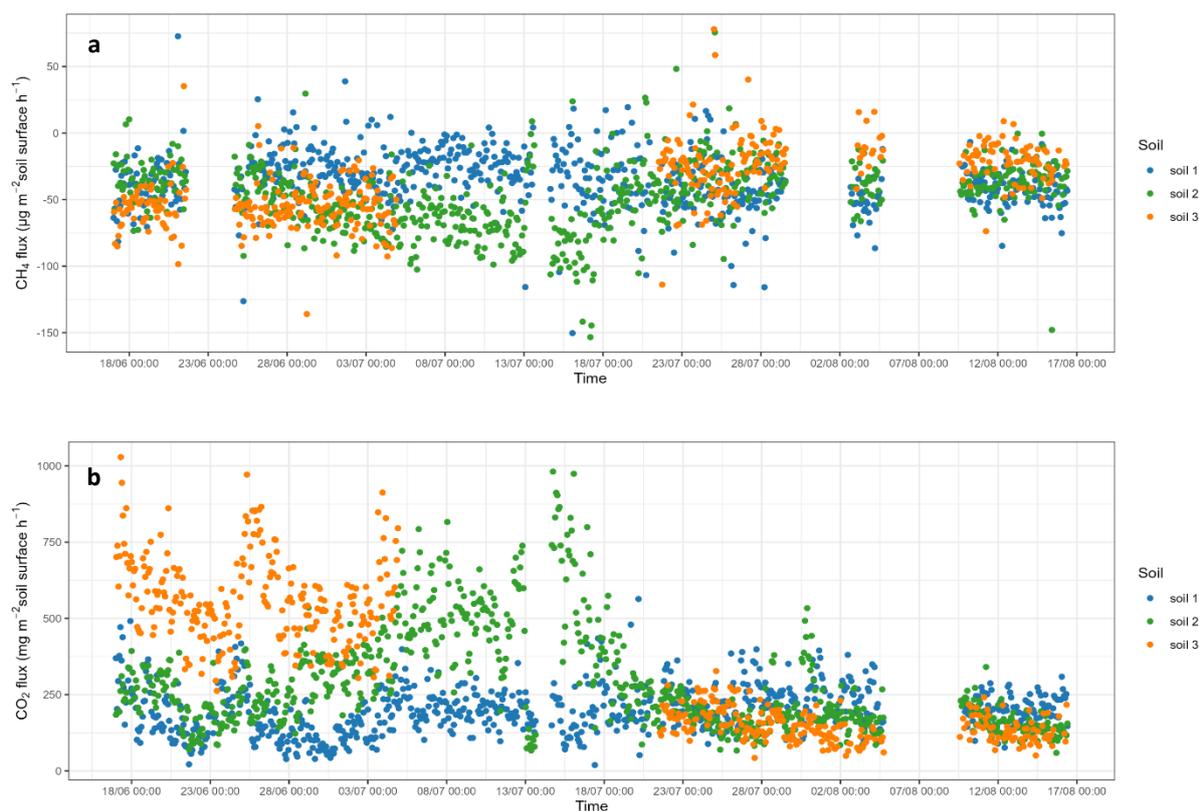


Figure 4. 5 Daily data of soil CH₄ (a) and CO₂ (b) fluxes close to each tree stand (soil collar 1, blue; soil collar 2, green; soil collar 3, orange) during 17th June to 16th August 2021 (60 days).

4.4.3 Diurnal patterns of stem and soil CH₄ and CO₂ fluxes

To observe the diurnal pattern of stem and soil CH₄ and CO₂ flux during the entire studying period, we used normalised gas flux data to remove the magnitude difference of CH₄ and CO₂ fluxes between dates (Fig. 4.6). While differences between the median fluxes in each 4-hour periods were small and there was substantial variation in the flux measurements, we observed a significant time of day effect ($P < 0.001$) on normalised stem CH₄ flux, but no significant time \times height interaction (Table 4.2). Normalised poplar stem CH₄ flux was significantly larger during the four hours after midnight (00:00-04:00) than during the morning and afternoon (04:00-16:00) ($P < 0.05$). Normalised poplar stem CH₄ flux was also significantly larger during the four hours before midnight (20:00-24:00) compared to that during the morning (08:00-12:00) ($P < 0.05$).

Normalised poplar stem CO₂ flux showed a large and significant diurnal pattern ($P < 0.001$, Table 4.2), and the diurnal pattern varied with measurement height (a significant time \times height interaction). At all three heights, stem CO₂ flux was significantly larger during the night-

time (20:00-04:00) than during the period of 04:00 to 16:00 ($P < 0.002$) with the smallest CO_2 flux during the morning (08:00-12:00).

We observed a small but significant diurnal effect on normalised soil CH_4 fluxes and a larger diurnal change on the CO_2 fluxes ($P < 0.001$, Table 4.3, Fig. 4.7). Soil CH_4 flux rates were significantly smaller during midnight (00:00-04:00) than during the afternoon (12:00-16:00) ($P = 0.021$). Soil CH_4 flux rates were significantly smaller from 04:00 to 08:00 compared to the period from 08:00 to 24:00 ($P < 0.01$). The diurnal pattern in soil CO_2 flux tended to be similar to that of the poplar tree stem CO_2 flux. Normalised soil CO_2 flux exhibited substantially larger CO_2 flux during the night-time and early morning (00:00-08:00) than during the period of 08:00 to 16:00 ($P < 0.001$); after that soil CO_2 flux started to increase again and soil CO_2 flux was significantly larger during the period of 16:00 to 24:00 than during the period of 08:00 to 16:00 ($P < 0.001$).

Table 4. 2 Linear mixed model results of the time of day and height effects of normalised stem CH_4 and CO_2 fluxes (4-hourly bins). Significant coefficients are highlighted in bold with the level of significance indicated: $p < 0.001$ (***) , $p < 0.01$ (**), $p < 0.05$ (*).

Type III Tests of Fixed Effects						
Source	Normalised stem CH_4 flux			Normalised stem CO_2 flux		
	df	F	P	df	F	P
intercept	1	0.381	0.537	1	3729.776	<0.001***
time	5	5.431	<0.001***	5	99.155	<0.001***
height	2	0.420	0.657	2	3.790	0.023*
time × height	10	1.450	0.152	10	2.063	0.024*

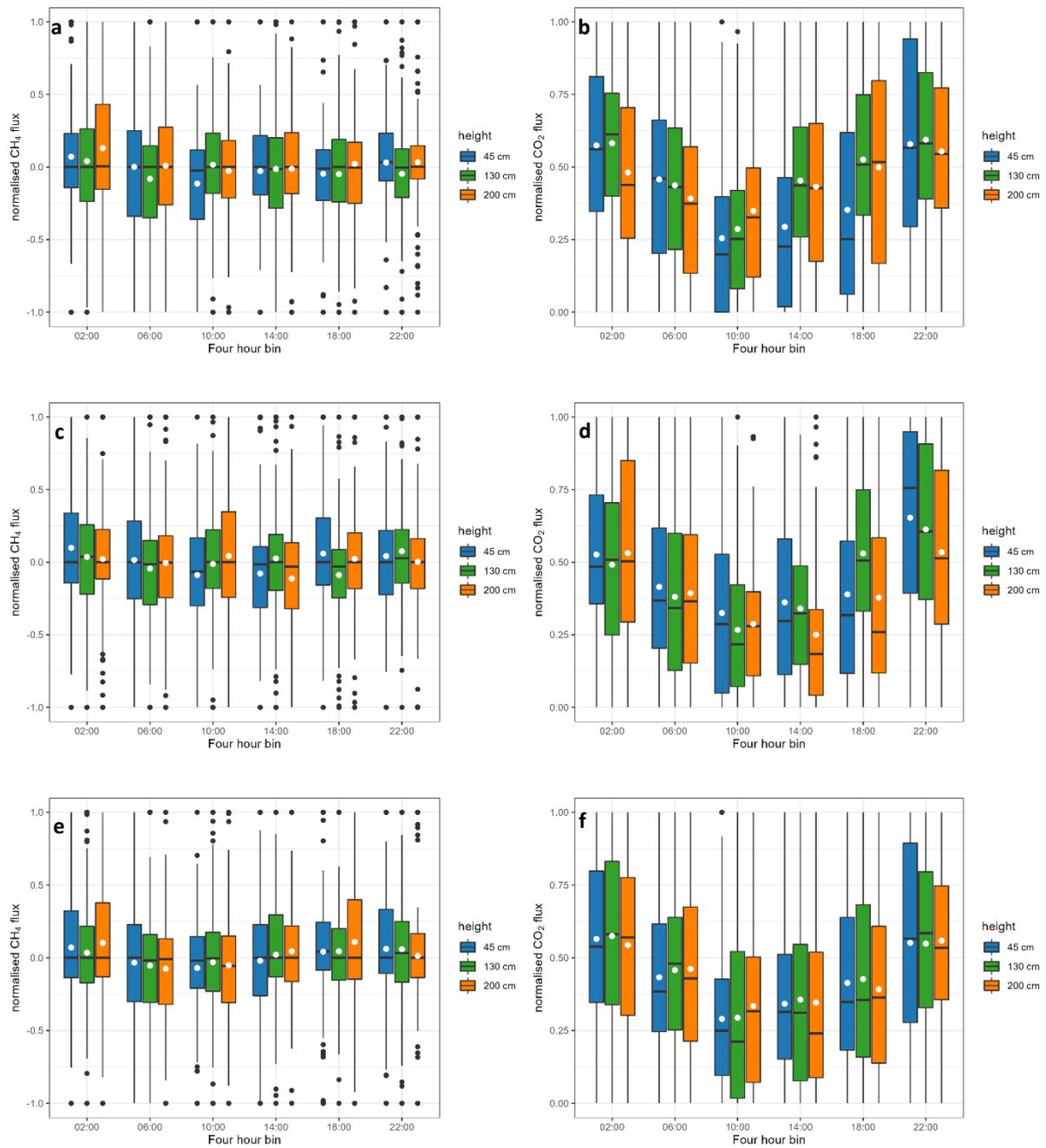


Figure 4. 6 Diurnal variation of three white poplar tree normalised stem CH₄ and CO₂ fluxes (tree 1: a, b; tree 2: c, d; tree 3: e, f) at 45 (blue), 130 (green) and 200 cm (orange) height in summer 2021. Data have been grouped into 4-hour 'bins'. For all boxplots in this study, the black line in the box represents the median and the interquartile range box represents the middle 50% of the data. The whiskers extend from either side of the box. The whiskers represent the ranges for the bottom 25% and the top 25% of the data values, excluding outliers. The outliers are shown in black dots and mean values are shown in white dots. A positive flux indicates emission and a negative indicates uptake.

Table 4. 3 Linear mixed model results of the time of day effect for normalised soil CH₄ and CO₂ fluxes (4-hourly bins). Significant coefficients are highlighted in bold with the level of significance indicated: $p < 0.001$ (***), $p < 0.01$ (**).

Type III Tests of Fixed Effects						
Source	Normalised soil CH ₄ flux			Normalised soil CO ₂ flux		
	df	F	P	df	F	P
intercept	1	457.620	0.002**	1	2723.359	<0.001***
time	5	5.729	<0.001***	5	37.659	<0.001***

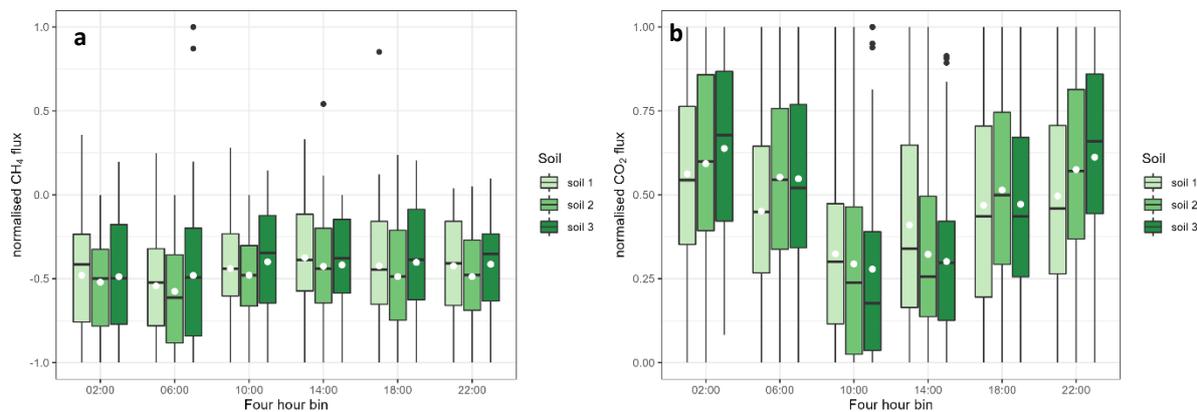


Figure 4. 7 Diurnal variation of normalised soil CH₄ (a) and CO₂ (b) fluxes close to each tree stand (soil collar 1, light green; soil collar 2, green; soil collar 3, dark green) in summer 2021. Data have been grouped into 4-hour 'bins'.

4.4.4 Stem height effects on stem CH₄ and CO₂ fluxes

We did not find a significant stem height effect on the normalised stem CH₄ flux ($P=0.657$, Table 4.2). However, we observed a significant height effect on the normalised stem CO₂ flux ($P=0.023$), and a significant time \times height interaction ($P= 0.024$; Fig. 4.6b, d, f; Table 4.2). The height effect on the normalised stem CO₂ flux was not consistent across the 4-hourly bins. During early evening (16:00-20:00), normalised CO₂ flux was significantly larger at 130 cm height than that at 45 cm height ($P<0.01$), which was particularly clear for tree 1 and tree 2, but this was not the case at other times of the day.

4.4.5 Diurnal patterns of environmental variables

Air temperature, soil temperature (at 10 cm depth) and volumetric soil moisture content (0-6 cm depth) all presented significant diurnal variation during the studying period ($P<0.001$, Fig. 4.8). Air temperature started to decrease during the night-time (20:00-04:00) with the

lowest value during the period of 04:00 to 08:00 ($P < 0.001$), and after that air temperature increased during the daytime (08:00-16:00) with the highest value during the period of 16:00 to 20:00 ($P < 0.001$). There was a lag difference between soil temperature and air temperature of around 4 hours. Soil temperature started to decrease from the midnight to early morning (00:00-08:00) with the lowest value during the period of 08:00 to 12:00 ($P < 0.001$), and after that soil temperature increased during the rest of the day (12:00-00:00). Soil moisture was higher during the period of 00:00 to 12:00 than the rest of the day ($P < 0.001$), and reached the lowest value during the evening (20:00-24:00) ($P < 0.001$).

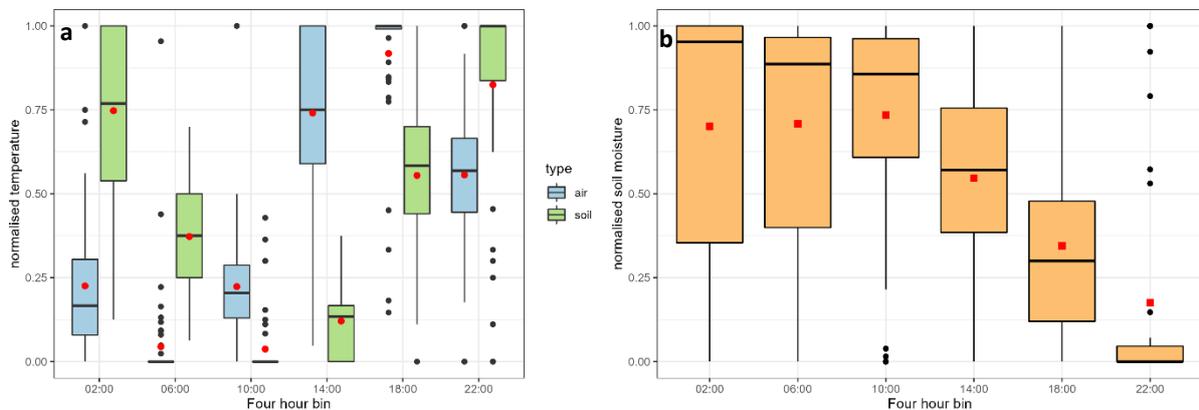


Figure 4. 8 Diurnal variation of normalised air (blue) and soil temperature (at 10 cm depth, green) (a) and soil moisture (0-6 cm depth, orange, b) in summer 2021. The mean value was shown in red dots. Data have been grouped into 4-hour ‘bins’.

The initial CH_4 concentration (at the start of gas flux measurements) ranging from 1.81 to 2.57 ppm also showed a large and significant diurnal pattern during the measurement period ($P < 0.001$, Fig. 4.9). The normalised stem initial CH_4 concentration was significantly higher during the midnight and early morning (00:00-08:00) than the rest of the day ($P < 0.001$) and reached the highest value during the period of 04:00 to 08:00 ($P < 0.001$). After that it decreased during the daytime with the lowest value during the period of 12:00 to 20:00 ($P < 0.001$) and then it started to increase again during the evening (20:00-24:00). We did not observe a significant stem height effect on initial CH_4 concentration ($P = 0.740$), nor a significant time \times height interaction ($P = 0.980$).

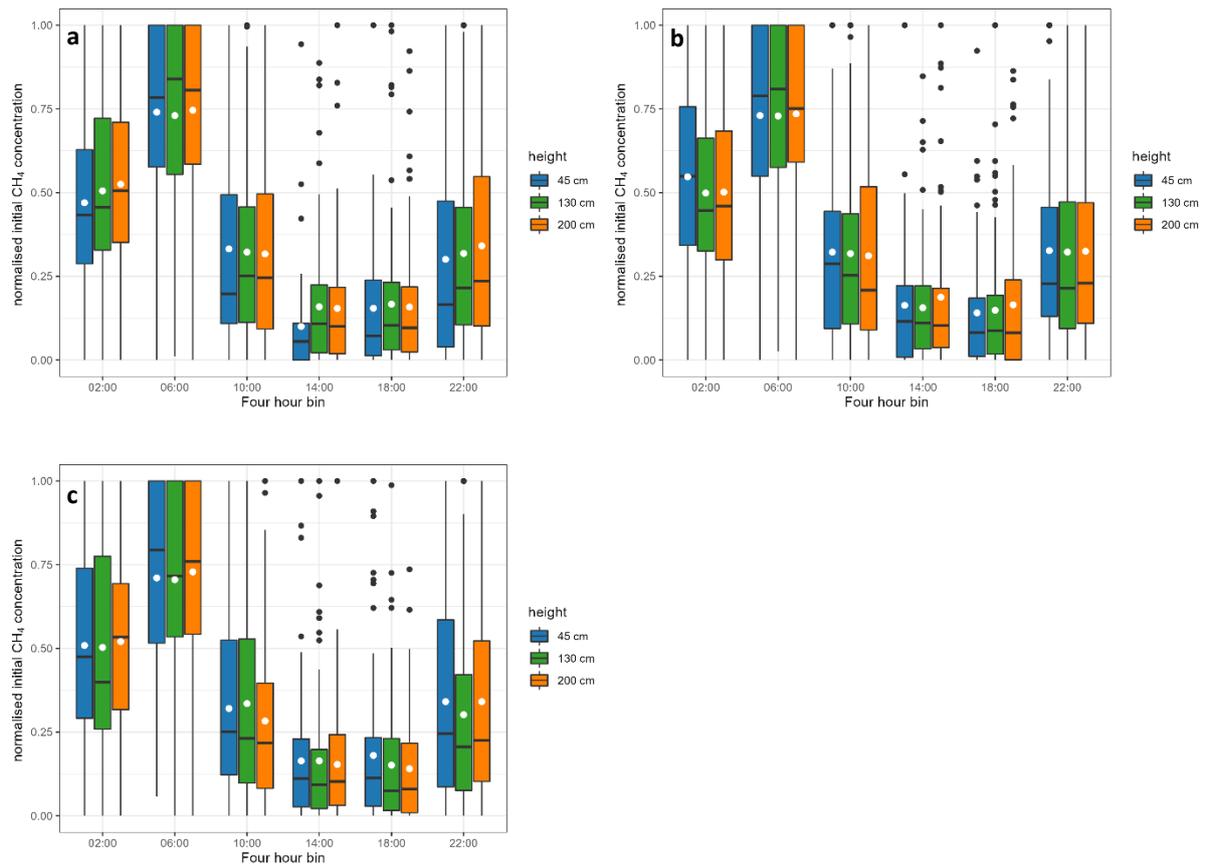


Figure 4. 9 Diurnal variation of three white poplar normalised initial CH₄ concentration (tree 1: a; tree 2: b; tree 3: c) at 45 (blue), 130 (green) and 200 cm (orange) height in summer 2021. Data have been grouped into 4-hour ‘bins’.

4.4.6 Correlations between normalised stem gas fluxes and potential drivers

We analysed the normalised four-hourly mean value of stem CH₄ and CO₂ fluxes at 45, 130 and 200 cm heights during the entire study period to determine their potential drivers (Table 4.4). Stem CH₄ flux at the three different stem heights did not show a significant correlation with soil moisture, initial CH₄ concentration or soil CH₄ flux. However, two of the trees showed a significant, positive correlation between the stem CH₄ flux and soil temperature at a stem height of 45 cm only ($P=0.034$ and 0.021), and one exhibited a significant, positive correlation between stem CH₄ flux and air temperature at 200 cm height ($P=0.009$). Interestingly, 7 out of the 9 correlations between stem CH₄ flux and stem CO₂ flux at 45, 130 and 200 cm heights were positive ($P<0.10$).

Stem CO₂ flux at 45, 130 and 200 cm heights for all three trees showed a significant, positive correlation with soil temperature ($P<0.001$). The correlations between stem CO₂ flux and soil moisture and air temperature were however not consistent. Only 3 of the 9 correlations

between stem CO₂ flux and air temperature were significant ($P < 0.01$), but all showed positive correlations with air temperature (Table 4.4). Similarly, for soil moisture, only 4 of the 9 correlations between stem CO₂ flux and soil moisture were significant ($P < 0.05$) and they were negative correlations (Table 4.4). Stem CO₂ flux at 45, 130 and 200 cm heights was significantly positively correlated to soil CO₂ flux at ($P < 0.05$), except for one tree stem at 200 cm height ($P = 0.804$).

Table 4. 4 Spearman's Rank-Order Correlation between normalised three white poplar tree stem (at 45, 130 and 200 cm height) and soil CH₄ and CO₂ fluxes and potential drivers throughout the entire study's data collection for each of the three individual trees. Significant coefficients are highlighted in bold with the level of significance indicated: p < 0.001 (***) , p < 0.01 (**), p < 0.05 (*), p < 0.1 (+).

Tree ID	height	stem gas flux	Spearman's Rank-Order Correlation													
			soil moisture		soil temperature		air temperature		initial CH ₄ concentration		soil CH ₄ flux		soil CO ₂ flux		stem CO ₂ flux	
			r _s (N)	P	r _s (N)	P	r _s (N)	P	r _s (N)	P	r _s (N)	P	r _s (N)	P	r _s (N)	P
Tree 1	45 cm	stem CH ₄ flux	0.001(203)	0.984	0.148(203)	0.034*	-0.071(203)	0.308	0.046(229)	0.484	-0.058(226)	0.387	-	-	0.244(230)	<0.001***
		stem CO ₂ flux	-0.057(222)	0.398	0.371(222)	<0.001***	-0.052(222)	0.439	-	-	-	-	0.295(263)	<0.001***	-	-
	130 cm	stem CH ₄ flux	0.088(257)	0.158	-0.017(257)	0.782	0.010(257)	0.872	-0.073(288)	0.217	0.061(280)	0.306	-	-	0.124(288)	0.035*
		stem CO ₂ flux	-0.162(281)	0.006**	0.426(281)	<0.001***	0.183(281)	0.002**	-	-	-	-	0.142(322)	0.011**	-	-
	200 cm	stem CH ₄ flux	0.024(251)	0.708	0.104(251)	0.100+	-0.021(251)	0.737	0.037(281)	0.531	0.033(272)	0.588	-	-	0.166(281)	0.005**
		stem CO ₂ flux	-0.274(273)	<0.001***	0.252(273)	<0.001***	0.160(273)	0.008**	-	-	-	-	0.014(313)	0.804	-	-
Tree 2	45 cm	stem CH ₄ flux	-0.082(222)	0.221	0.155(222)	0.021*	-0.051(222)	0.446	0.044(251)	0.482	0.007(251)	0.906	-	-	0.094(252)	0.135
		stem CO ₂ flux	-0.133(246)	0.036*	0.338(246)	<0.001***	-0.064(246)	0.313	-	-	-	-	0.218(293)	<0.001***	-	-
	130 cm	stem CH ₄ flux	-0.007(251)	0.917	0.038(251)	0.545	-0.040(251)	0.522	-0.035(281)	0.560	-0.089(280)	0.138	-	-	0.132(282)	0.026*
		stem CO ₂ flux	-0.228(274)	<0.001***	0.434(274)	<0.001***	0.222(274)	<0.001***	-	-	-	-	0.239(322)	<0.001***	-	-
	200 cm	stem CH ₄ flux	0.100(232)	0.126	-0.020(232)	0.760	-0.046(232)	0.482	-0.010(259)	0.866	0.093(262)	0.132	-	-	0.201(262)	0.001**
		stem CO ₂ flux	-0.101(252)	0.108	0.432(252)	<0.001***	-0.023(252)	0.713	-	-	-	-	0.261(299)	<0.001***	-	-
Tree 3	45 cm	stem CH ₄ flux	-0.127(229)	0.053+	0.081(229)	0.222	0.118(229)	0.075+	-0.032(259)	0.605	0.015(179)	0.839	-	-	0.112(260)	0.071+
		stem CO ₂ flux	-0.009(252)	0.886	0.281(252)	<0.001***	0.017(252)	0.789	-	-	-	-	0.208(222)	0.002**	-	-
	130 cm	stem CH ₄ flux	-0.047(222)	0.481	0.093(222)	0.164	0.068(222)	0.312	-0.017(252)	0.785	-0.049(175)	0.520	-	-	0.088(252)	0.162
		stem CO ₂ flux	-0.047(243)	0.463	0.326(243)	<0.001***	-0.040(243)	0.531	-	-	-	-	0.267(215)	<0.001***	-	-
	200 cm	stem CH ₄ flux	-0.098(209)	0.155	0.077(209)	0.264	0.179(209)	0.009**	-0.085(273)	0.191	-0.111(146)	0.178	-	-	0.191(238)	0.003**
		stem CO ₂ flux	-0.120(229)	0.069+	0.272(229)	<0.001***	0.025(229)	0.705	-	-	-	-	0.253(184)	<0.001***	-	-

4.5 Discussion

4.5.1 Significance of high within-day variation in stem CH₄ and CO₂ fluxes

The mean tree stem CH₄ flux over two months at our research site was relatively small, as higher hour-to-hour net CH₄ emission and net CH₄ uptake rates largely offset each other. The high-frequency measurements of tree stem CH₄ fluxes from mid-June to mid-August at 45, 130 and 200 cm above the soil were 0.66 ± 21.63 , 0.29 ± 20.95 and 0.93 ± 19.57 $\mu\text{g m}^{-2}$ stem surface h^{-1} , respectively. The results were much smaller than the high-frequency measurements of a hickory (*Carya cordiformis*) tree during April to July in a temperate upland forest, with a mean value of 26.50 and 16.13 $\mu\text{g m}^{-2}$ stem surface h^{-1} at 75 and 150 cm above the soil, respectively (Barba, Poyatos and Vargas, 2019). However, the high-frequency measurements provided evidence of the large variation in the magnitude of stem CH₄ flux in both studies. In our study, we captured 7124 observations of three poplar tree stem CH₄ flux at three stem heights (45, 130 and 200 cm above the soil) during the entire experimental period (60 days). Barba, Poyatos and Vargas (2019) reported that at least 45 measurements were required in order to provide more accurate stem CH₄ flux during the whole period (100 days). Compared to the manual measurements of tree stem CH₄ flux (monthly frequency), the automated high-frequency (hourly frequency) measurements play a vital role in studying the temporal pattern of stem CH₄ flux by capturing the high variability of flux magnitude and potential underlying mechanisms (Barba et al., 2018, 2021). At our research site, all three measured poplar trees at all stem heights showed high variability of stem CH₄ flux magnitude with 51.1% stem CH₄ uptake and 48.9% CH₄ emission over the entire experimental period. The results of both tree stem CH₄ uptake and emission made it more complicated to understand the underlying mechanism of tree stem CH₄ exchange in temperate upland forests.

Similar to stem CH₄ flux, only a few studies reported the high-frequency measurements of tree stem CO₂ flux in temperate upland forests. The high-frequency measurements of poplar tree stem CO₂ fluxes from mid-June to mid-August at 45, 130 and 200 cm above the soil were 98.13 ± 50.57 , 91.76 ± 47.06 and 72.55 ± 40.27 mg m^{-2} stem surface h^{-1} , respectively. The results were much smaller than the high-frequency measurements of a hickory (*Carya cordiformis*) tree during April to July in a temperate upland forest, with a mean value of 753.98

and 448.27 mg m⁻² stem surface h⁻¹ at 75 and 150 cm above the soil, respectively (Barba, Poyatos and Vargas, 2019).

In our study, we observed a large within-day variation in stem CH₄ and CO₂ fluxes over the 60-day period. The overall impact of these large within-day variations plays an important role in estimating seasonal stem CH₄ and CO₂ fluxes and their overall contribution to forest greenhouse gas fluxes. Based on our results, measuring at different times of the day is essential for the seasonal estimates of stem CH₄ and CO₂ fluxes. The observations of large hour-to-hour variations in stem CH₄ flux, and the smaller stem CO₂ flux during daytime than during night-time (discussed below), strongly indicate the importance of high-frequency (hourly frequency) measurements of tree stem CH₄ and CO₂ fluxes during both daytime and night-time. Future studies are required to determine the stem CH₄ and CO₂ fluxes using the high-frequency (hourly frequency), within-day measurements in different forest ecosystems on a global scale, to obtain a more accurate estimate of the contribution of stem gas fluxes to the overall greenhouse gas budget of forests.

4.5.2 Hour-to-hour variation in tree stem CH₄ and CO₂ fluxes

In our study, both stem CH₄ oxidation and production occurring were observed and the net balance between the two processes varied greatly from hour to hour. Similar to our results, Barba, Poyatos and Vargas (2019) using high-frequency measurement (hourly frequency) also reported that tree stem CH₄ flux exhibited a large daily coefficient of variation during the whole period (100 days) in a temperate upland forest, but tree stems mainly acted as a net CH₄ source. The simultaneously occurring stem CH₄ emission and CH₄ uptake at our research site complicate the understanding of the origin of tree stem CH₄ flux.

Tree stem height can be used as an indicator for the origin of stem CH₄ emission, e.g. if stem CH₄ emissions decrease with increasing stem height, this suggests the source of the CH₄ is from biological soil CH₄ production. However, we did not observe a stem height effect on tree stem CH₄ flux (P=0.657) during the whole period. In contrast, high-frequency measurements observed that the mean value of a hickory tree stem CH₄ fluxes was larger at 75 cm stem height than those at 150 cm stem height in a temperate upland forest during April to July (Barba, Poyatos and Vargas, 2019). However, stem CH₄ flux did not decrease significantly with increasing tree stem heights in three hickory trees at the same research site during April to

December (Barba et al., 2021). Furthermore, we did not find any significant correlations between CH₄ flux in three poplar tree stems at each stem height and soil CH₄ flux at our research site (Table 4.4), and soils almost always were a net CH₄ sink during the whole period and no potential CH₄ production was found in the 0-50 cm soil profile (unpublished data, R. Ma). Studies have reported that tree stem CH₄ emissions declined with increasing stem height in wetland forests, suggesting tree stem CH₄ source from deeper layers of anaerobic soils (Terazawa et al., 2007; Pangala et al., 2013, 2014, 2017; Jeffrey et al., 2019; Jeffrey et al., 2021b). Compared to wetland, the origin of tree stem CH₄ production in temperate upland forests is most likely to be inside the tree stem itself (Wang et al., 2016, 2017; Barba et al., 2021). Studies have found methanogenic communities inside the heartwood and sapwood of poplar (*Populus deltoides* and *Populus* sp.) trees in both temperate and subtropical upland forests (Yip et al., 2018; Feng et al., 2022). Stem lenticel density can also affect stem and root aerenchyma tissues and diffusion pathways, which may change the tree-mediated CH₄ emissions (Pangala et al., 2014). However, we did not observe a significant difference in lenticel density between tree heights (P=0.586). To understand the potential CH₄ production and oxidation of three trunk layers (bark, sapwood and heartwood) and the role of stem lenticel presence, wood incubation experiments may provide further evidence of the origin of tree stem CH₄ flux (Barba et al., 2019; Chapter Five).

Surprisingly, we observed stem CH₄ uptake occurred for a large proportion of time at each stem height (50-51% of measurements). The lack of stem height effect on stem CH₄ flux in our study may be explained by stem CH₄ uptake and the net soil CH₄ sink. Although most studies only reported tree stem CH₄ emissions by manual measurements in temperate upland forests (Wang et al., 2016; Warner et al., 2017; Pitz and Megonigal, 2017; Pitz et al., 2018), we observed both tree stem CH₄ uptake and emissions from English oak (*Quercus robur*) and Japanese larch (*Larix kaempferi*) in a temperate upland forest (Chapter Three). In addition, recent studies have shown that novel CH₄ oxidising monooxygenases were detected from Norway spruce (*Picea abies*) shoots in boreal upland forests (Putkinen et al., 2021), and the observation of methane oxidising bacteria inside the heartwood and sapwood of poplar (*Populus* sp.) in a subtropical upland forest (Feng et al., 2022) and inside the bark of *Melaleuca quinquenervia* in subtropical lowland forests (Jeffrey et al., 2021a) provides further evidence of CH₄ uptake inside tree stems. In addition, methane oxidising bacteria within lowland tree

stems exhibited a novel CH₄ sink, which oxidised around one-third of stem CH₄ flux derived from soil (Jeffrey et al., 2021b). Further studies are required to identify methane oxidising bacteria and their community abundance of tree stems in temperate upland forests by molecular techniques.

At our research site, although we did not find a stem height effect on stem CH₄ flux, a significant height effect on stem CO₂ flux was observed ($P=0.023$). Contrary to our hypothesis, stem CO₂ flux was larger at 130 cm above the soil than that of 45 and 200 cm. However, the height effect on stem CO₂ flux was not consistent within the three individual trees at each time interval, although individual tree as a random factor only explained 17.6% ($P=0.537$) of the variance of stem CO₂ flux in the linear mixed model. In contrast, other studies found stem CO₂ flux decreased with tree height on hickory (*Carya cordiformis*) trees in a temperate upland forest, but the results were only based on two heights with the separation of 75-100 cm (Barba, Poyatos and Vargas, 2019; Barba et al., 2021). It was reported that the stem height effect on Norway spruce (*Picea abies* (L.) Karst.) stem CO₂ flux varied seasonally, which showed the largest stem CO₂ flux at 18 m compared to 1.3 and 10 m height in summer and no difference between heights during non-growing seasons over 3 years in a boreal upland forest (Tarvainen, Röntfors and Wallin, 2014). There are two sources of stem CO₂ flux, i.e. the respiring cells in the stem and roots, and the rhizosphere (Teskey et al., 2007). Although we observed a significant positive correlation between stem CO₂ flux at 45, 130 and 200 cm heights and soil CO₂ flux ($P<0.05$, Table 4.4), which may be due to the effect of similar environmental drivers such as soil temperature, the stem CO₂ originated within the tree stem itself could be the major source. As CO₂ concentration inside the stem is normally considerably larger than in soil, the diffusion gradient is from the root into soil rather than the opposite route (Teskey et al., 2007; 2017). The inconsistent stem height influence on stem CO₂ flux within three individual poplar trees may be due to the difference in resistance to radial CO₂ diffusion. It was reported that in three manipulated poplar (*Populus deltoides* Bartr. ex Marsh) trees stem CO₂ flux was linearly correlated to xylem CO₂ concentration, but the differences in physical barriers to stem CO₂ diffusion were tree-specific and thus the variation in stem CO₂ diffusion to the atmosphere was due to local respiring cells and transported CO₂ (Steppe et al., 2007). Further studies are needed to understand and model the stem CO₂ flux

at different stem heights with the consideration of measuring the diffusion coefficients and internal xylem CO₂ concentration.

4.5.3 Diurnal patterns of tree stem CH₄ and CO₂ fluxes

Over the experimental period (60 days), high-frequency measurements showed only a small but significant diurnal effect on poplar stem CH₄ flux ($P < 0.001$). Similar to our results, other studies using automated high-frequency (hourly frequency) measurements also reported diurnal variations in tree stem CH₄ flux in temperate upland forests (Pitz and Megonigal, 2017; Barba, Poyatos and Vargas, 2019). However, manual measurement (2-4 weeks frequency) of grey alder (*Alnus incana* (L.) Moench) did not show any significant difference in stem CH₄ flux between daytime (12:00-16:00) and night-time (00:00-04:00) in a temperate riparian forest (Schindler et al., 2021). The lack of diurnal pattern of stem CH₄ flux may be due to the limited sampling frequency and a number of observations of stem gas fluxes on an hourly basis, which further emphasised that high-frequency measurements are more sensitive to capture the hotspots and temporal pattern of tree stem CH₄ flux. Contrary to our hypothesis, we found stem CH₄ flux at three heights was significantly larger during the midnight (00:00-04:00) than that during dawn and afternoon (04:00-08:00, 08:00-12:00 and 12:00-16:00), and compared to the evening (20:00-24:00), stem CH₄ flux was significantly smaller during the morning (08:00-12:00) ($P < 0.05$). In contrast, a single tree (*Liriodendron tulipifera*) at 75 cm above the soil showed stem CH₄ flux peaked in the late afternoon (16:20) during a 3-day measurement, which was linked to sap flux density (Pitz and Megonigal, 2017). The diurnal measurements of poplar trees (*Liriodendron tulipifera* L., *Populus deltoides* Bartr. ex Marsh.) showed nearly zero value of sap flow rates during the night-time and early morning (23:00-08:00) and increasing sap flow rates during daytime (08:00-18:00) and decreasing sap flow rates in the evening (18:00-23:00) (McLaughlin, Wullschleger and Nosal, 2003; Steppe et al., 2007). Although we did not measure the sap flow rates in our study, the observation of larger stem CH₄ flux during the night-time than in the daytime may suggest that larger stem CH₄ fluxes are not simply associated with higher sap flow rates. However, another study reported that although sap flow density and stem temperature explained 14% and 10% of the diurnal variation in stem CH₄ flux for specific days, the correlations between diurnal stem CH₄ flux and its drivers were not consistent during the whole growing season (Barba, Poyatos and Vargas, 2019).

Although all the environmental variables we measured followed a clear diurnal trend, it is still complicated to understand the potential drivers of tree stem CH₄ flux based on the results we found. At our research site, we did not observe any significant correlation between stem CH₄ flux and soil moisture and initial CH₄ concentration at three different heights, and the relationship between stem CH₄ flux and air and soil temperature was not consistent within individual trees and at different heights. It was suggested the clear diurnal variation in tree stem CH₄ flux may be linked to pressurized ventilation or transpiration-driven mass flow from soil-derived CH₄ production (Covey and Megonigal, 2019). However, the large proportion of stem CH₄ uptake, combining the lack of stem height effect and the non-significant correlation between stem CH₄ flux with soil CH₄ flux, made it harder to draw the conclusion that the source of stem CH₄ flux is mainly from biological soil CH₄ production.

The diurnal pattern of poplar stem CH₄ flux we found in our study might also be explained by the temporal variability of stem CH₄ oxidation. A recent study reported that the average CH₄ oxidation rates of two lowland *Melaleuca quinquenervia* tree stems were twice higher at afternoon sampling points (13:00 and 16:00) than those of the dawn sampling points (08:00) (Jeffrey et al., 2021b). Jeffrey et al. (2021b) have discussed the potential drivers of this temporal pattern of CH₄ oxidation in the paper, including the effect of temperature, sap-flux and oxygen gradients which can affect the activity and metabolism of methanotrophs, the changes in soil produced CH₄ source due to rhizosphere oxidation during photosynthesis, the decline in stem water content during transpiration, and the difference between diffusive gas transport (night-time and daytime) and active transpiration gas transport (daytime only). In addition, the diurnal pattern of yellow poplar (*Liriodendron tulipifera* L.) stem shrinkage during daytime and expansion during night-time which was negatively correlated with sap flow rates may cause the difference in diffusive gas transport between daytime and night-time (Mclaughlin, Wullschleger and Nosal, 2003). However, in our study we did not find a diurnal air and soil temperature-driven differences driving stem CH₄ flux, and CH₄ derived from the soil is unlikely to be the major source of tree stem CH₄ flux at our research site. Further research is required to study the diurnal pattern of stem CH₄ flux by measuring stem water content and stem increment, and comparing the passive diffusive gas transport and active transpiration-driven mass flow using isotopic techniques in temperate upland forests. On the other hand, identifying biological *in situ* stem CH₄ production and oxidation from the

stem itself needs further microbiological techniques, such as metagenomic tools (Putkinen et al., 2021). Interestingly, 7 out of 9 automated measurements from our results showed positive correlations between stem CH₄ and CO₂ fluxes ($P < 0.10$, Table 4.4) over the whole period. The correlations may be attributed to gas diffusivity heterogeneity through the wood (Barba et al., 2019), which might share a similar pattern of axial diffusion from the stem interior by common physical barriers, but this relationship did not mean stem CH₄ and CO₂ fluxes were originating from a similar source (Magonigal, Brewer and Knee, 2020). In addition, similar to the results published by Barba et al. (2021), the correlation coefficients between poplar stem CH₄ and CO₂ fluxes at each height were small ($r_s = 0.124-0.244$) at our research site, which implies that several drivers are likely to be involved in the diurnal pattern of stem CH₄ flux.

Over the experimental period (60 days), high-frequency measurements showed a large significant diurnal effect on poplar stem CO₂ flux ($P < 0.001$). Contrary to our hypothesis, all three poplar tree stems exhibited larger CO₂ flux during the night-time (20:00-04:00) than the rest of the day, and CO₂ flux decreased within the daytime with the smallest during the morning (08:00-12:00), after that CO₂ flux started to increase again during the afternoon and evening (12:00-20:00). Consistent with our results, other researchers reported a similar diurnal pattern of tree stem CO₂ flux among various tree species (*Populus deltoides* Bartr. ex Marsh, *Quercus alba* L. and *Liriodendron tulipifera* L.), with stem CO₂ flux increasing during the night-time and decreasing during the daytime (Teskey and Mcguire, 2002; Steppe et al., 2007). Their results showed that the stem CO₂ flux was linearly related to xylem CO₂ concentration by both laboratory and field measurements, which exhibited the opposite diurnal pattern of stem sap flow (Teskey and Mcguire, 2002; Steppe et al., 2007; Aubrey and Teskey, 2021). Although soil respiration showed a similar diurnal pattern as the stem CO₂ flux at our research site (Fig. 4.7b), we did not observe decreased stem CO₂ flux with increasing stem height (Fig. 4.6b, d, f), which suggested that stem and roots respiration were more responsible for the emission of stem CO₂ flux to the atmosphere. Similar to stem CH₄ flux, the diurnal pattern of stem CO₂ flux with the largest value during the night-time is difficult to be linked to sap flow rate, as CO₂ normally does not move upward via sap flow in the dark due to low sap flow rates (Teskey and Mcguire, 2002, 2007). It was believed that respiratory CO₂ can dissolve and be transported via transpiration stream at night and thus cause the

release of CO₂ from the stems to the atmosphere (Steppe et al., 2007). Further study using labeled ¹³CO₂ supported this hypothesis, which showed that CO₂ diffusion from tree (*Populus deltoids*) stem and branch to the atmosphere was estimated up to 83-94% by root-respired CO₂ via the transpiration stream (Bloemen et al., 2013). The decrease of stem CO₂ flux during the daytime in our study may be due to the increasing sap flow rates, which diluted respiratory CO₂ inside the xylem (Teskey and McGuire, 2007; Steppe et al., 2007). Contrary to tree stem CH₄ flux, we observed soil temperature exhibited a similar diurnal pattern as tree stem CO₂ flux (Fig. 4.8a) and positively correlated with all three poplar tree stem CO₂ flux at 45, 130 and 200 cm heights (P<0.001, Table 4.4). Similar results were found that the diurnal variation of tree (*Quercus robur* L.) stem CO₂ flux was positively correlated to stem temperature (Saveyn, Steppe and Lemeur, 2007).

Temperature may play a vital role in stem CO₂ flux due to its effect on the rate of respiration and solubility of gases (Teskey and McGuire, 2007). However, it was indicated that temperature cannot be the only factor to accurately predict stem CO₂ flux, as the diurnal fluctuation of stem CO₂ flux was observed when air temperature was kept constant (Steppe et al., 2007). It was suggested that the diurnal variation of the dissolved CO₂ in the xylem may depend on a mix of abiotic and biotic factors, such as sap flow rate, sap pH, transpiration rate, nutrient uptake, and production and turnover of root and root-associated organisms and all the factors that influence those processes (Aubrey and Teskey, 2021). Due to the positive relationship between stem CO₂ flux and xylem CO₂ concentration (Teskey and McGuire, 2002; Steppe et al., 2007), the environmental factors that may have an impact on dissolved CO₂ in the xylem could also affect the diurnal variation in stem CO₂ flux. However, the effect of those various environmental factors on the diurnal pattern of stem CO₂ flux is poorly understood due to the difficulty in distinguishing between each factor. Combined with measurements of stem CH₄ flux, further studies are required to determine the sap flow rate and transpiration rate driving the diurnal pattern of stem CH₄ and CO₂ fluxes under both laboratory manipulation and field *in situ* experiments.

4.6 Conclusion

High-frequency measurements of stem CH₄ and CO₂ fluxes were taken at 45, 130 and 200 cm above the soil in three white poplar trees from mid-June to mid-August (60 days) in a

temperate upland woodland. The large variation in the magnitude of stem CH₄ flux was captured by high-frequency measurements, which showed around half of the observations (51.1%) were stem CH₄ uptake among all three measured poplar trees at all stem heights over the entire experimental period. We did not observe a stem height effect on tree stem CH₄ flux, which may be explained by the large proportion of tree stem CH₄ uptake at each stem height and the net soil CH₄ sink at our research site. Stem CO₂ flux was larger at 130 cm above the soil than at 45 and 200 cm. However, the stem height effect on stem CO₂ flux was not consistent within three individual trees at each time interval, which may be due to the difference in resistance to radial CO₂ diffusion. Stem CH₄ flux showed a small but significantly diurnal effect, while stem CO₂ flux exhibited a clearer diurnal pattern during the entire experimental period. We found stem CH₄ flux at three heights was significantly larger during midnight than that during dawn and afternoon. There were no significant correlations between stem CH₄ flux and soil CH₄ flux, soil moisture and initial stem CH₄ concentration at three different stem heights. Stem CO₂ flux was larger during the night-time than the daytime, and was positively correlated with soil temperature. Due to high temporal variability in tree stem CH₄ and CO₂ fluxes, high-frequency measurements are required for the accurate estimation of stem gas fluxes and their contribution to overall greenhouse gas budgets in forests.

5. Potential CH₄ Production and Oxidation Rates of Bark, Sapwood and Heartwood from *Populus Alba* via Wood Incubation

5.1 Abstract

Recent studies have reported CH₄ emission and uptake from tree stems in forests. To date, limited knowledge has been developed on the underpinning processes. Wood incubation experiments may help to confirm the capacity of wood CH₄ production and oxidation. However, few studies have performed and quantified the potential CH₄ production and oxidation rates of all trunk layers. Stem lenticel acts as a major pathway of O₂ entry, which may have an influence on the microbial communities inhabited in bark and thus affect potential wood CH₄ production and oxidation rates. In this study, we aimed to determine the effect of trunk layers and lenticel presence on potential wood CH₄ production and oxidation rates of white poplar (*Populus alba*) trees under anaerobic and aerobic conditions. The results showed that potential wood CH₄ production and oxidation rates were significantly different between trunk layers (P=0.009 and 0.016), which bark exhibited significantly higher CH₄ production and oxidation rates than those from sapwood and heartwood (P<0.05). However, we did not observe any significant effect of lenticel presence on potential CH₄ production and oxidation via incubation (P=0.154 and 0.621). Internal *in situ* CH₄ concentration was close to or even lower than the atmospheric CH₄ concentration during two campaigns of wood core sampling, with an average of 1.35 ± 0.25 and 1.87 ± 0.97 ppm within 48 h, respectively. In contrast, substantial CO₂ concentration inside wood cores was observed, reaching 1-2 orders of magnitude higher than the atmospheric CO₂ concentration. Consistent with previous high-frequency measurements of poplar stem CH₄ emission and uptake, wood incubation experiments further confirmed the capacity of CH₄ production and oxidation from bark, sapwood and heartwood.

5.2 Introduction

In recent years, novel findings of tree stem CH₄ emissions in boreal, temperate and tropical forests suggest that they may contribute to the uncertainty of global CH₄ budget estimates (Machacova et al., 2016, 2021; Pangala et al., 2017; Jeffrey et al., 2019; Barba et al., 2019, 2021; Covey and Megonigal, 2019; Bréchet et al., 2021; Saunois et al. 2020). Basically, there

are mainly two assumptions about the underlying mechanisms of CH₄ emissions from tree stems. Studies reported that in floodplain and wetland forests, CH₄ is biologically produced in anoxic saturated soils or dissolved in groundwater, and then absorbed by roots and transported in stems through intercellular spaces and aerenchyma tissue via the transpiration stream, and finally diffused by tree stem to the atmosphere (Terazawa et al., 2007, 2015; Rice et al., 2010; Gauci et al., 2010; Pangala et al., 2013, 2015, 2017; Jeffrey et al., 2019; Jeffrey et al., 2021b; Sakabe et al., 2021). However, it was believed that tree stem CH₄ can also be produced biologically *in situ* inside the heartwood in temperate forests on mineral soils where soil is a net CH₄ sink (Covey et al., 2012; Wang et al., 2016, 2017; Yip et al., 2018; Barba et al., 2021).

The capacity of potential CH₄ production from bark-to-pith wood cores under anaerobic incubation in the lab further provided the evidence of biologically *in situ* stem CH₄ production (Covey et al., 2012; Pangala et al., 2017). The anatomy of a tree trunk includes three layers, i.e. bark, sapwood and heartwood. Compared to bark and sapwood, heartwood of *Populus davidiana* showed the highest potential CH₄ production rate during 48 h anaerobic incubation at 20°C (Wang et al., 2016). Similar results were observed from *Carya cordiformis* with higher CH₄ production capacity in the heartwood than in the sapwood (Barba et al., 2021). However, the anaerobic incubation of *Carya cathayensis* Sarg. only exhibited low potential CH₄ production rates of three trunk layers (0.0003-0.0014 µg CH₄ g⁻¹ DW h⁻¹) (Wang et al., 2016). The results indicate that potential wood CH₄ production depends on tree species and trunk layers. To date, only one study reported the capacity of potential CH₄ oxidation from all three trunk layers of *Populus davidiana* and *Carya cathayensis* Sarg. under aerobic incubation, but the CH₄ oxidation rates were undetectable (Wang et al., 2016). However, recent studies discovered methanotrophs from bark, sapwood and heartwood of tree species in subtropical forests (Jeffrey et al., 2021a; Feng et al., 2022) provide further evidence of CH₄ oxidation inside tree stems. Further research is required to investigate the wood capacity for CH₄ production and oxidation under anaerobic and aerobic conditions via incubation experiments, in order to understand the underlying mechanism of stem CH₄ exchange.

Lenticels are lens-shaped in the periderm, which are produced by intercellular spaces. Stem lenticels mostly develop beneath stomata and act as a key role in transpiration and water and gases exchange (Langenfield-Heyser, 1997). The diameter and length of pore channels in

lenticel phellogen of *Alnus glutinosa* (L.) Gaertn were reported at c. 1µm and 12 µm (Buchel and Grosse, 1990). Stem lenticel density of flooded *Alnus glutinosa* saplings positively controlled stem CH₄ emissions via the transport of soil-produced CH₄, which suggested that stem lenticels were the exit for tree stem CH₄ emissions (Pangala et al., 2014). Trees in upland forests normally do not develop aerenchyma and form hypertrophied lenticels (Langenfeld-Heyser, 1997), but the presence of lenticels on tree stems is a major pathway of O₂ entry (Dittert, Wötzel and Sattelmacher, 2006). The communities of methanogens and methanotrophs inhabited inside the bark with the presence of stem lenticels may differ due to the higher exposure to O₂. However, no studies have reported the capacity of wood CH₄ production and oxidation from tree cores with stem lenticel presence.

This study therefore aimed to determine the potential CH₄ production and oxidation rates in the three main layers of trunks (bark, sapwood and heartwood) and from areas with or without lenticels of white poplar (*Populus alba*) trees at different heights, using lab incubation experiments.

The hypotheses were:

1. All trunk layers have the capacity to produce CH₄ under anaerobic conditions, and heartwood has the highest potential CH₄ production rate.
2. All trunk layers have the capacity to oxidise CH₄ under aerobic conditions, and bark has the highest potential CH₄ oxidation rate.
3. Bark from wood cores with the presence of stem lenticel exhibits lower potential CH₄ production and higher CH₄ oxidation rates than those from sapwood and heartwood.

5.3 Methods

5.3.1 Study site

The research was carried out in a managed, planted woodland on a mineral soil, located on the campus of the University of York, United Kingdom, at 53°56'38"N, 0°03'29"W (UK Grid Reference SE619501). The soil is a well-draining, sandy clay loam with a pH of 7.2. Mean annual maximum and minimum temperatures were 13.6 °C and 5.7 °C, respectively, and the annual total precipitation was 603.2 mm (1981 to 2010, data from Church Fenton station, located 17.6 km southwest of the forest (UK Met Office Library & Archive; www.metoffice.gov.uk)). The research plot was dominated by white poplar (*Populus alba*)

scattered with a few horse chestnut (*Aesculus hippocastanum*) and alder (*Alnus glutinosa*). Three white poplar trees were selected for this study (Table 5.1).

Table 5. 1 The characteristics of selected white poplar trees (n=3 trees) in woodland at the campus of the University of York

Type of shedding leaves	Tree species	Common name	DBH ^a (cm) Mean ± SD	Tree height (m) Mean ± SD	Tree anatomy
Deciduous broadleaf	<i>Populus alba</i>	white poplar	35.3 ± 4.7	25.4 ± 1.1	diffuse- to semi-ring-porous

^aDBH: Diameter at breast height

5.3.2 Experimental design and bark/wood sampling

We studied potential wood CH₄ and CO₂ production under anaerobic conditions and wood CH₄ oxidation and CO₂ production under aerobic conditions. Three trunk layers i.e. bark, sapwood and heartwood of white poplar stems were examined, from areas with or without lenticels at 45, 130 and 200 cm stem height. The incubation experiments were performed for 48 h at 20 °C in the lab (Fig. 5.1).

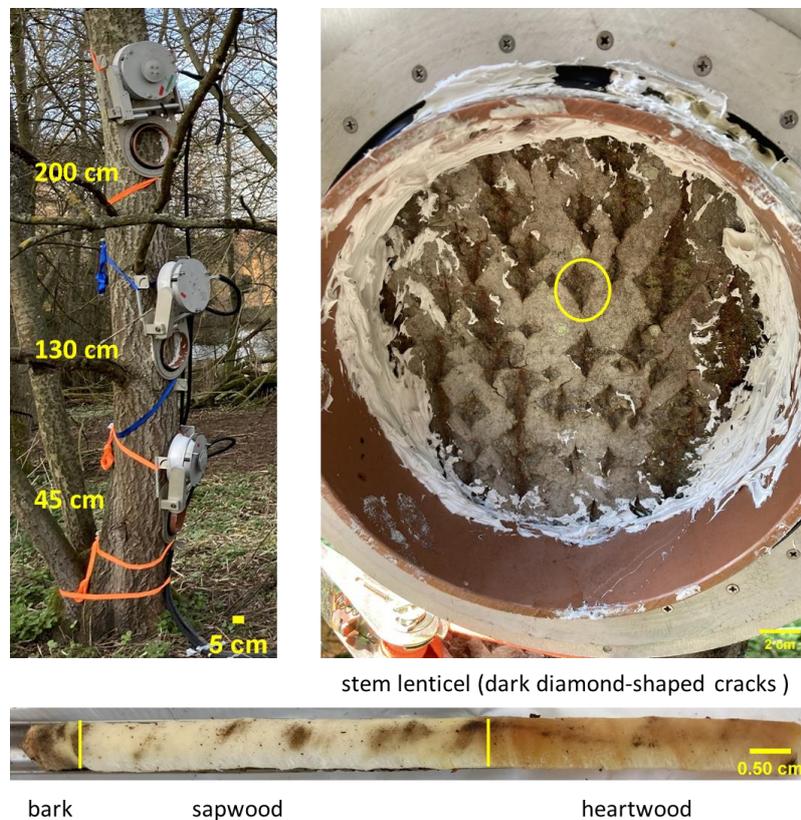


Figure 5. 1 Pictures of the treatments of the wood incubation experiments.

White poplar wood samples were collected on 16th August 2021 for anaerobic incubation and 18th August 2021 for aerobic incubation. They were collected within the collars at 45, 130 and 200 cm heights used for the high-frequency measurements of gas fluxes of the three poplar trees (Chapter 4). Tree cores (72 in total) were collected using an increment borer (5.15 mm internal diameter, 300 mm length, two screws, Haglöf Sweden, Längsele, Sweden), which was drilled into the pith (Fig. 5.2). For each incubation condition (anaerobic or aerobic), 4 tree cores were taken inside each PVC collar, perpendicular to the length of the tree stem: two cores, each in the center of a lenticel, and two cores, each in an area between lenticels. The increment borer was sanitized with ethanol between samples and before use. Each core was split into bark, sapwood and heartwood depending on depth and coloration. The length and diameter of wood samples were measured using a ruler or a calliper, respectively. The two sub-replicate cores in each PVC collar were immediately put together in one pre-weighed 12-mL sterile Exetainers (Labco, High Wycombe, UK) for incubation. All the wood samples were returned to the laboratory within 1 h of collection. For the anaerobic incubations, each wood sample inside the 12 ml Exetainer was flushed with N₂ for 2 min before closing the lid of the Exetainer with a butyl rubber stopper in the lab. For the aerobic incubations, each wood sample was stored inside the 12 ml Exetainer and left in the field for 15 min to establish ambient air headspace conditions and they were then sealed with the lid of the Exetainer.

After drilling the wood core, we immediately plugged the hole in the tree with a 11-mm stopper (SubaSeal, Sigma-Aldrich, Sigma-Aldrich Company Ltd., UK). Each stopper was wrapped in Parafilm (Bemis, Parafilm M Laboratory) to enable a gas-tight seal (Fig. 5.2). *In situ* CH₄ and CO₂ concentration in the headspace of each hole was determined at 0, 24 and 48h by sampling 20 ml of gas from the headspace using a 20 mL syringe, storing the sample into pre-evacuated 12-mL Exetainer and analysing it on a gas chromatography (see below). After finishing measurements, all the holes were sealed with non-VOC sealant (Marmox Multibond Adhesive Sealant, Zero VOC, ecomerchant, UK) to avoid potential disease.



Figure 5. 2 The increment borer was drilled inside the white poplar tree and the holes were plugged with 11 mm stoppers attached with Parafilm (left). The stem lenticels are dark diamond-shaped cracks (right).

5.3.3 Wood incubation

Wood incubation experiments were performed in the lab under both anaerobic and aerobic conditions in darkness at 20 °C using a method adapted from Toet et al. (2017). Pilot tests were carried out first to determine the incubation time required for the potential CH₄ production and oxidation rate. Wood cores (n=9 in total) were taken from a nearby white poplar tree, split into bark, sapwood and heartwood and six wood cores were measured at 0, 24, 48, 120 and 192 h for anaerobic incubation, and another three wood cores were measured at 0, 24 and 120 h for aerobic incubation. No significant differences in potential CH₄ production and oxidation rates over the full length of 8 days or 5 days during anaerobic and aerobic incubation of the pilot experiments (P>0.05). In the pilot experiment for aerobic incubation, we also determined the effect of the CH₄ concentration in the exetainer on the potential CH₄ oxidation rates by using the initial CH₄ concentration of 5 ppm during a 0-120 h incubation period and found the starting CH₄ concentration of 5 ppm was not the limiting concentration for CH₄ oxidation.

For the anaerobic incubations, five empty control Exetainers were also flushed with N₂ for 2 min in the lab before closing the lid of the Exetainer with a butyl rubber stopper. Two min after adding another 2 ml of N₂ to each Exetainer, 2 ml of headspace was sampled from each Exetainer (0 h) and stored in 3 ml evacuated Exetainers to which 5 ml of N₂ was added. The headspace was sampled the same way at 24 and 48 h over a 2-day incubation. For the aerobic incubations, five control Exetainers with wood samples were similarly equilibrated for 15 min

with ambient air in the field. During gas sampling at T0, 2 ml of air with concentrated CH₄ was added to the exetainer, to create a start concentration of 5 ppm CH₄ in the headspace. Then 2 ml of headspace was sampled 2 min after adding the CH₄ from each Exetainer (0 h) and injected in a 3 ml evacuated Exetainers to which 5 ml of N₂ was added. During the sampling after 24 and 48 h, 2 ml of outside air at 24 and 48 h was first added to the exetainers. The rates of potential CH₄ production and oxidation, and the rates of potential CO₂ emission under anaerobic and aerobic conditions were determined by a PerkinElmer-Arnel gas chromatography (GC, AutoSystem XL, PerkinElmer Instruments, Shelton, CT, USA) equipped with a flame ionization detector (FID) and a 3.7 m Porapak Q 60/80 mesh column.

The *in situ* internal CH₄ and CO₂ concentrations taken at 0, 24 and 48h were analysed on the gas chromatograph (described above). To determine the gravimetric moisture content of the bark and wood samples at the start of the incubation, fresh bark and wood samples within 1 hour of the collection were weighed. Bark and wood samples after incubation were dried at 70°C for 24 h to determine the dry mass (Yip et al., 2019). Wood volume was calculated by the length and diameter of wood samples (Williamson & Wiemann, 2010) (Appendix 2).

5.3.4 Potential wood gas production or oxidation rates calculation

The calculation of potential CH₄ production and oxidation rates, and CO₂ emission rates followed the equations described below:

Step 1 - Calculate CH₄ or CO₂ concentration (ppm) from 3 ml Exetainer

$$C_{gas} = (A_{gas} - A_{blank}) / (A_{ref} - A_{blank}) \times C_{ref} \quad \text{Equation 1}$$

where C_{gas} = CH₄ or CO₂ concentrations (ppm), A_{gas} = peak area of CH₄ or CO₂ (μV·s), A_{blank} = peak area of CH₄ or CO₂ inside N₂ (μV·s), A_{ref} = peak area of reference gas (μV·s) and C_{ref} = reference gas concentration, which CH₄ is 100 ppm and CO₂ is 505.5 ppm.

Step 2 - Convert CH₄ or CO₂ concentration (ppm) into (mg CH₄ L⁻¹ or mg CO₂ L⁻¹) from 3 ml Exetainer

$$F_{gas} = (C_{gas} \times M_{gas}) / (R \times T \times 10^3) \quad \text{Equation 2}$$

where F_{gas} = CH₄ or CO₂ concentrations (mg CH₄ L⁻¹ or mg CO₂ L⁻¹), M_{gas} = molar mass of CH₄ or CO₂ (g mol⁻¹), R = gas constant, which is 0.082057 L atm mol⁻¹ K⁻¹ and T is incubation Kelvin temperature (K).

Step 3 - Calculate the amount of CH₄ or CO₂ in 3 ml Exetainer

$$m_{gas} = [F_{gas} \times 7 \times 10^{-3} \times (7/2)] - [F_{blank} \times 5 \times 10^{-3} \times (5/7)] \quad \text{Equation 3}$$

where m_{gas} = CH₄ or CO₂ mass (mg CH₄ or mg CO₂), F_{blank} = CH₄ or CO₂ concentrations inside N₂ (mg CH₄ L⁻¹ or mg CO₂ L⁻¹).

Step 4 - Calculate the amount of CH₄ or CO₂ in 12 ml Exetainer

For anaerobic incubation:

$$m_{gas,final} = [(m_{gas} \times 14/12) - (F_{blank,N2} \times 2 \times 10^{-3} \times (2/14))] \times (12/2) \quad \text{Equation 4}$$

For aerobic incubation:

$$m_{gas,final} = [(m_{gas} - (F_{blank,air} \times 2 \times 10^{-3} \times (2/14))] \times (12/2) \quad \text{Equation 5}$$

where $m_{gas,final}$ = CH₄ or CO₂ mass (mg CH₄ or mg CO₂), F_{blank} = CH₄ or CO₂ concentrations inside N₂ or inside air (mg CH₄ L⁻¹ or mg CO₂ L⁻¹).

5.3.5 Statistical analysis

All the statistical tests were performed in SPSS Statistics Software (Version 28; IBM Corp.). The gas production and oxidation rates and internal gas concentrations met the assumptions of homogeneity of variance (Levene's test) and normality (Kolmogorov-Smirnov test) and internal CH₄ and CO₂ concentrations we used log transformation to reduce heteroscedasticity. Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality. We chose potential CH₄ and CO₂ production and CH₄ oxidation rates from 24-48 hours, rather than 0-24 hours for further data analysis. As after the disturbance of sampling, the methanogens and methanotrophs did not respond quickly within the first 24 h incubation. We performed a three-way Analysis of Variance (ANOVA) test to assess the effect of trunk layer, the presence of lenticel and stem height on potential CH₄ and CO₂ production and oxidation rates. Due to the observed significant height × layer and lenticel × height interaction effects on potential CO₂ production rates under aerobic incubation, a two-way ANOVA test was conducted to test for trunk layer, stem height and

lenticel presence effects on CO₂ production rates. We performed linear mixed effects analysis to assess the presence of lenticel and stem height on internal CH₄ and CO₂ concentrations over 48 h. The fixed factors lenticel presence, tree height and incubation time (and their interactions) and the random factor individual tree were included in the model. Due to the observed significant lenticel × height interaction effect on internal CO₂ concentration, a two-way ANOVA test was conducted to test for stem height and lenticel presence effects on CO₂ concentration. Posthoc tests (Tukey) were applied if a significant effect (P<0.05) was found.

5.4 Results

5.4.1 The effect of trunk layer on potential wood CH₄ and CO₂ production and oxidation rates

We examined potential CH₄ and CO₂ production and oxidation rates of bark, sapwood and heartwood under anaerobic and aerobic conditions, respectively (Table 5.2&3, Fig. 5.3&4). We observed a significant trunk layer effect on potential CH₄ production rates (P=0.009, Table 5.2), with bark exhibiting significantly higher CH₄ production rates than those from sapwood (P=0.028) and heartwood (P=0.019). The mean potential CH₄ production rates of bark, sapwood and heartwood were 0.017 ± 0.028 (mean ± SD), 0.001 ± 0.002 and 0.001 ± 0.001 μg CH₄ g⁻¹ DW h⁻¹, respectively (Fig. 5.3a). Similar to anaerobic incubation, we observed a significant trunk layer effect on potential CH₄ oxidation rates (P=0.016, Table 5.3), which bark exhibited significantly higher CH₄ oxidation rates than those from sapwood (P=0.045) and heartwood (P=0.026). The mean potential CH₄ oxidation rates of bark, sapwood and heartwood were 0.012 ± 0.022, 0.001 ± 0.003 and 0.000 ± 0.001 μg CH₄ g⁻¹ DW h⁻¹, respectively (Fig. 5.4a).

Table 5. 2 Three-way ANOVA results of lenticel, trunk layer and height effect of potential CH₄ and CO₂ production rates under anaerobic incubation. Significant coefficients are highlighted in bold with the level of significance indicated: p < 0.001 (***), p < 0.01 (**), p < 0.05 (*), p < 0.10(+).

Tests of Between-Subjects Effects						
Source	potential CH ₄ production rate			potential CO ₂ production rate		
	df	F	P	df	F	P
intercept	1	7.361	0.010**	1	207.780	<0.001***
lenticel	1	2.122	0.154	1	0.153	0.698
height	2	0.393	0.678	2	1.304	0.284
layer	2	5.327	0.009**	2	138.915	<0.001***
lenticel × height	2	0.467	0.630	2	2.584	0.089+
lenticel × layer	2	2.071	0.141	2	1.124	0.336
height × layer	4	0.202	0.936	4	1.125	0.360
lenticel × height × layer	4	0.411	0.800	4	2.207	0.088+

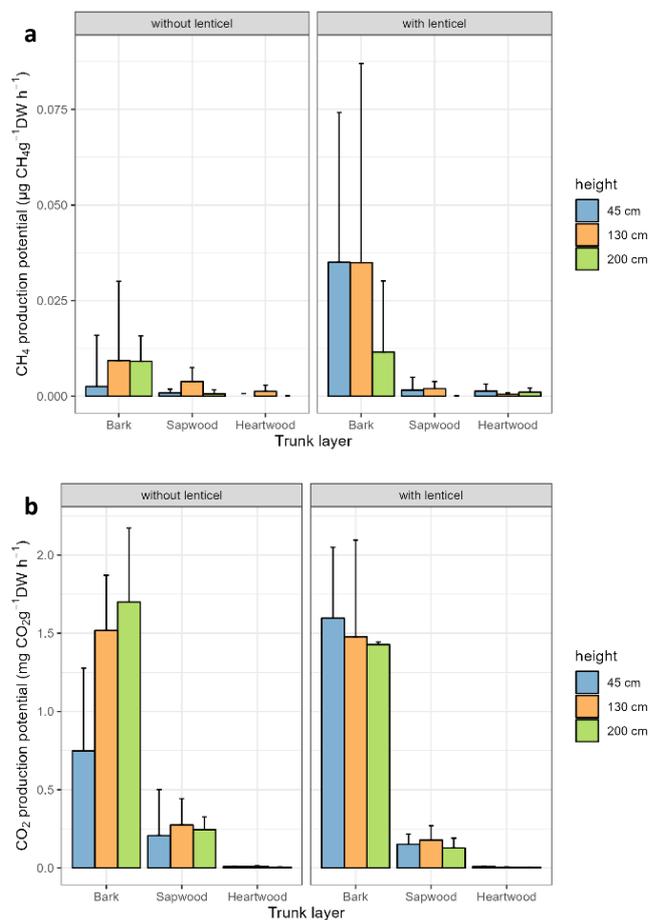


Figure 5. 3 Potential CH₄ (a) and CO₂ (b) production rate of three trunk layers with and without lenticels at 45 (blue), 130 (orange) and 200 cm (green) height during wood incubation under anaerobic conditions. Positive value of potential CH₄ production rate represents net CH₄ emission.

Analogous to potential CH₄ production rates, we observed a significant effect of trunk layer on potential CO₂ production rates under anaerobic conditions (P < 0.001, Table 5.2). Bark

showed significantly higher potential CO₂ production rates than those of sapwood and heartwood (P<0.001). The mean potential anaerobic CO₂ production rates of bark, sapwood and heartwood were 1.411 ± 0.494, 0.197 ± 0.138 and 0.007 ± 0.003 mg CO₂ g⁻¹ DW h⁻¹, respectively (Fig. 5.3b). We observed a significant effect of the trunk layer on aerobic potential CO₂ production rates (P<0.001, Table 5.3). We also observed significant lenticel x height, height x layer and lenticel x height x layer on potential aerobic CO₂ production rates (P<0.01, Table 5.3). Trunk layer showed a significant effect on the potential CO₂ production rate at each height (45, 130 and 200 cm), with higher rates in the bark than in the sapwood and heartwood (P<0.001). The mean potential aerobic CO₂ production rates of bark, sapwood and heartwood were 1.527 ± 0.441, 0.257 ± 0.110 and 0.012 ± 0.006 mg CO₂ g⁻¹ DW h⁻¹, respectively (Fig. 5.4b).

Table 5. 3 Three-way ANOVA results of lenticel, trunk layer and height effect of potential CH₄ oxidation and CO₂ production rates under aerobic incubation. Significant coefficients are highlighted in bold with the level of significance indicated: p < 0.001 (***), p < 0.01 (**), p < 0.05 (*).

Tests of Between-Subjects Effects						
Source	potential CH ₄ oxidation rate			potential CO ₂ production rate		
	df	F	P	df	F	P
intercept	1	6.901	0.013*	1	603.257	<0.001***
lenticel	1	0.249	0.621	1	0.068	0.796
height	2	1.843	0.173	2	5.901	0.006**
layer	2	4.700	0.016*	2	372.497	<0.001***
lenticel x height	2	0.806	0.455	2	7.646	0.002**
lenticel x layer	2	0.608	0.550	2	0.225	0.800
height x layer	4	1.083	0.380	4	3.991	0.009**
lenticel x height x layer	4	1.386	0.259	4	7.970	<0.001***

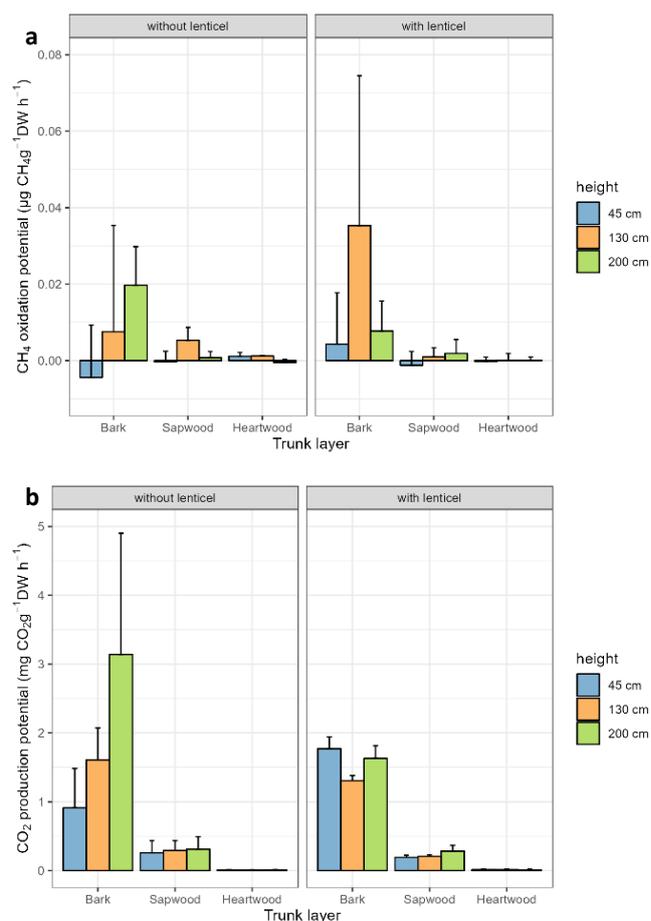


Figure 5.4 Potential CH₄ oxidation (a) and CO₂ production (b) rates of three trunk layers with and without lenticels at 45 (blue), 130 (orange) and 200 cm (green) height during wood incubation under aerobic conditions. Positive values of potential CH₄ oxidation rate represent net CH₄ uptake and negative values of potential CH₄ oxidation rate represent CH₄ emission.

5.4.2 The effect of lenticel presence and stem height on potential wood CH₄ and CO₂ production and oxidation rates

We also assessed the potential CH₄ and CO₂ production and oxidation rates in areas with lenticels or without and at different stem heights (Table 5.2&3, Fig. 5.3&4). However, the presence of lenticel, stem height and their interactions did not show any significant effects on potential CH₄ production and oxidation rates or potential anaerobic CO₂ production rates ($P > 0.05$, Table 5.2&3). However, we observed a significant effect of height, height × layer and lenticel × height on potential aerobic CO₂ production rates ($P < 0.01$, Table 5.3). A height effect was only found in bark with a higher potential aerobic CO₂ production rate at 200 cm than that of 45 cm ($P = 0.045$). In addition, a height effect on potential aerobic CO₂ production rate was only found in areas between lenticels ($P = 0.006$), which showed lower aerobic CO₂

production rates at 130 cm than at 45 and 200 cm height ($P=0.009$ and 0.020). Compared to without lenticels, we found the potential aerobic CO_2 production rate was significantly higher with lenticels at 45 cm ($P=0.020$), but the rate was significantly lower with lenticels at 200 cm ($P=0.015$).

5.4.3 Internal *in situ* CH_4 and CO_2 concentrations

The measurements of internal *in situ* CH_4 and CO_2 concentrations in the holes left after wood core sampling were taken immediately after sampling (T0) and after 24 and 48 hours during two campaigns (Table 5.4&5, Fig. 5.5&6). During the first campaign (Table 5.4), incubation time showed significant effect on internal CH_4 concentration ($P<0.001$), with significantly higher CH_4 concentrations at T24 and T48 than those at T0 ($P<0.001$). However, we did not observe any differences between cores in lenticel areas and between lenticel areas ($P=0.144$), and stem height did not show any significant effect on internal CH_4 concentration ($P=0.445$). Similar to internal CH_4 concentration, cores in lenticel areas and between lenticel areas did not show any significant differences in internal CO_2 concentration either ($P=0.416$). However, height showed a significant effect ($P<0.001$), with internal CO_2 concentrations being significantly higher at 130 cm than at 45 and 200 cm ($P<0.001$). During the second campaign (Table 5.5), we observed that the internal CH_4 concentration of cores in lenticel areas was significantly higher ($P=0.004$), but no height effect was found ($P=0.217$). The interaction effect of height and lenticel presence on cores showed a significant effect on internal CO_2 concentration ($P<0.001$). Internal CO_2 concentrations of cores between lenticel areas were significantly higher at 200 cm than those at 45 and 130 cm ($P<0.001$). Compared to cores between lenticel areas, we found internal CO_2 concentration of cores in lenticel areas was significantly higher at 45 cm ($P=0.008$), but CO_2 concentration of cores in lenticel areas was significantly lower at 200 cm ($P=0.021$). The internal CH_4 concentration was close to the atmospheric CH_4 concentration, with an average of 1.35 ± 0.25 and 1.87 ± 0.97 ppm within 48 h for the first and second campaigns, respectively (Fig. 5.5a, 5.6a). Compared to internal CH_4 concentration, the internal CO_2 concentration exhibited large variability between each tree core. The average internal *in situ* CO_2 concentrations within 48 h were 5290.00 ± 5139.48 and 9704.47 ± 10825.02 ppm for the first and second campaigns, respectively (Fig. 5.5b, 5.6b).

Table 5. 4 Linear mixed model results of incubation time, lenticel presence and stem height effect of internal CH₄ and CO₂ concentration during 16th-18th August 2021. Significant coefficients are highlighted in bold with the level of significance indicated: p < 0.001 (***) , p < 0.01 (**) , p < 0.05 (*) , p < 0.10 (+).

Tests of Between-Subjects Effects						
Source	internal CH ₄ concentration			internal CO ₂ concentration		
	df	F	P	df	F	P
Intercept	1	9216.560	<0.001***	1	6021.455	<0.001***
time	2	18.176	<0.001***	2	0.233	0.793
lenticel	1	2.175	0.144	1	0.668	0.416
height	2	0.817	0.445	2	12.884	<0.001***
time × lenticel	2	0.278	0.758	2	0.052	0.949
time × height	4	1.017	0.403	4	0.345	0.847
lenticel × height	2	1.937	0.150	2	2.519	0.086+
time × lenticel × height	4	0.452	0.770	4	0.167	0.955

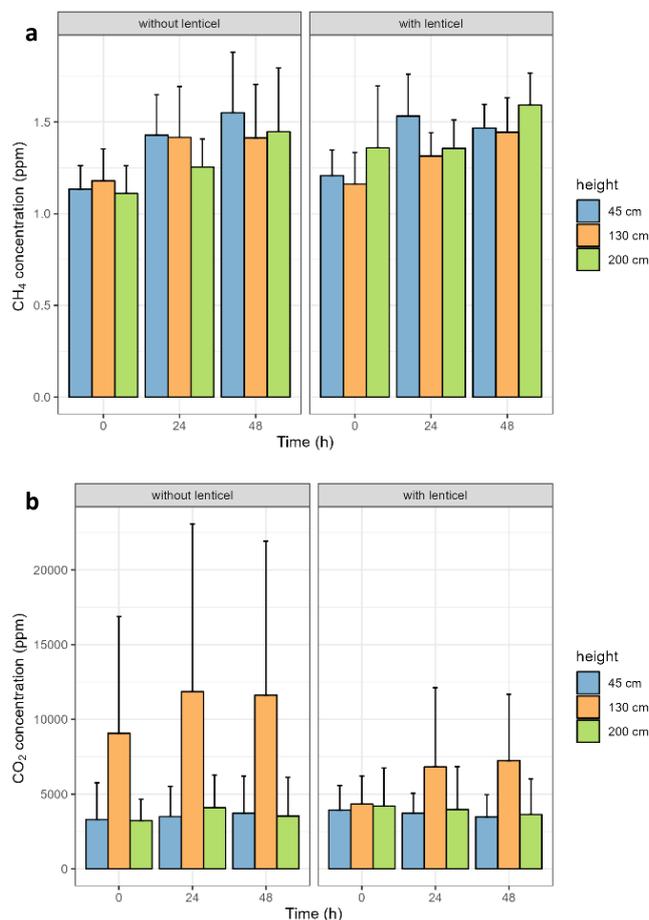


Figure 5. 5 Internal *in situ* CH₄ (a) and CO₂ (b) concentration with and without lenticels at 45 (blue), 130 (orange) and 200 cm (green) height for 48h on 16th-18th August 2021.

Table 5. 5 Linear mixed model results of incubation time, lenticel presence and stem height effect of internal CH₄ and CO₂ concentration during 18th-20th August 2021. Significant coefficients are highlighted in bold with the level of significance indicated: p < 0.001 (***), p < 0.01 (**), p < 0.05 (*), p < 0.10 (+).

Tests of Between-Subjects Effects						
Source	internal CH ₄ concentration			internal CO ₂ concentration		
	df	F	P	df	F	P
Intercept	1	672.719	0.001***	1	775.273	0.001***
time	2	0.071	0.932	2	1.610	0.206
lenticel	1	8.548	0.004**	1	3.363	0.070+
height	2	1.556	0.217	2	2.112	0.127
time × lenticel	2	0.122	0.885	2	0.279	0.757
time × height	4	0.044	0.996	4	0.723	0.578
lenticel × height	2	1.014	0.367	2	12.010	<0.001***
time × lenticel × height	4	0.153	0.961	4	0.408	0.802

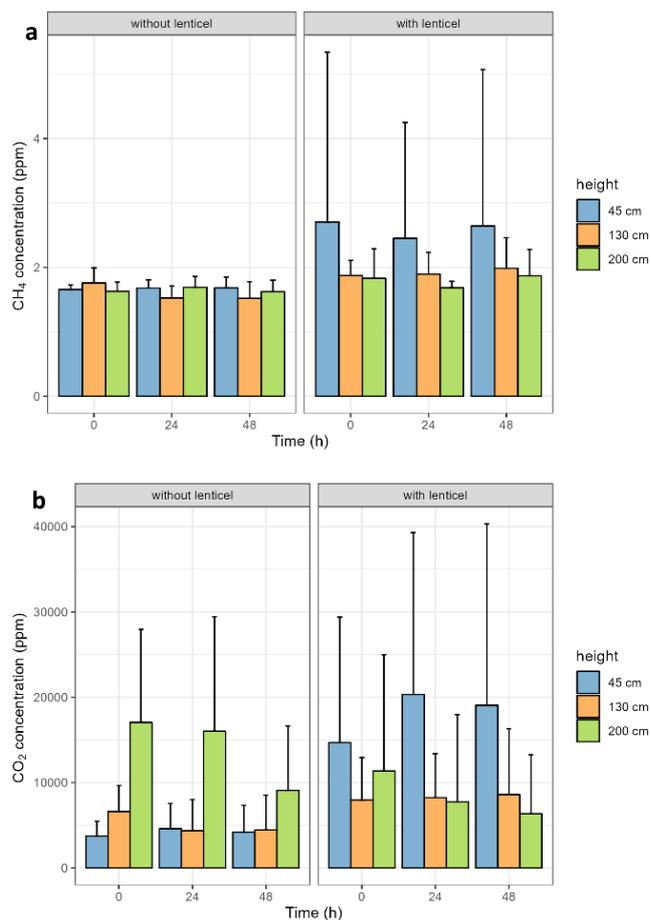


Figure 5. 6 Heartwood *in situ* CH₄ (a) and CO₂ (b) concentration with and without lenticels at 45 (blue), 130 (orange) and 200 cm (green) height for 48h on 18th-20th August 2021.

5.5 Discussion

5.5.1 The role of trunk layer on stem CH₄ production and oxidation

In our study, we observed significantly higher CH₄ production and oxidation rates from bark than those from sapwood and heartwood of white poplar (*Populus alba*) stems at 45, 130 and 200 cm height. Contrary to our hypothesis, heartwood of *Populus alba* did not show the highest potential CH₄ production rate. In contrast, Wang et al. (2016) reported that the highest potential CH₄ production rates of *Populus davidiana* were observed inside the heartwood (0.0378 μg CH₄ g⁻¹ DW h⁻¹) rather than bark (0.0014 μg CH₄ g⁻¹ DW h⁻¹) and sapwood (0.0003 μg CH₄ g⁻¹ DW h⁻¹) during 48 h dark anaerobic incubation at 20°C. However, the potential bark, sapwood and heartwood CH₄ oxidation rates of *Populus davidiana* were undetectable. Heartwood water content and wood density may explain the differences in the results. The heartwood water content of *Populus davidiana* at breast height was c. 64% and wood density was 0.34 g DW cm⁻³ (Wang et al., 2016). However, the heartwood water content of *Populus alba* at 130 cm in our study was 51.4% ± 2.8 and wood density was 0.45 ± 0.04 g DW cm⁻³ at our research site. Results showed that the potential heartwood CH₄ production rate of *Populus canadensis* was positively correlated to heartwood water content, especially when heartwood water content was above c. 60% (Li et al., 2020). High water content can enhance the activity of methanogens with anoxic conditions, while low wood density makes it easier for CH₄ diffusion (Wang et al., 2016). However, we did not observe any significant correlations between potential CH₄ production and oxidation rates of bark, sapwood and heartwood and wood water content or wood density (P>0.05), and there was no significant difference of wood water content and wood density between bark and heartwood of wood samples for both anaerobic and aerobic incubation (P>0.05). In our study, the potential CH₄ production rates of *Populus alba* from heartwood were almost 40 times lower than those from the heartwood of *Populus davidiana* reported by Wang et al. (2016). It was suggested that heartwood water content was not the limiting factor for tree species without substantial heartwood CH₄ production (Li et al., 2020). Current knowledge on the drivers of potential wood CH₄ production and oxidation is still limited, further studies are required to assess drivers (e.g. wood anatomy) of potential CH₄ production and oxidation within three trunk layers of different tree species.

Our previous results using high-frequency measurements showed both stem CH₄ uptake and CH₄ emission from white poplar trees at 45, 130 and 200 cm above the soil (Chapter Four). The wood incubation results indicate that CH₄ production and CH₄ oxidation can potentially occur in particular in the bark, and to a lower extent in the sapwood and heartwood. Studies have found that methanogenic communities are present in the heartwood and sapwood of several poplar species (*Populus deltoids*, *Populus* sp. and *Populus canadensis*) in both temperate and subtropical upland forests (Yip et al., 2018; Li et al., 2020; Feng et al., 2022). However, to date no study has reported methanogens associated with the bark. Future research is required to identify methanogenic communities inside and on the bark of different tree species using molecular techniques. In addition, the observation of methane oxidising bacteria inside the heartwood and sapwood of poplar trees (*Populus* sp.) in a subtropical upland forest (Feng et al., 2022) and from the bark of *Melaleuca quinquenervia* in subtropical lowland forests (Jeffrey et al., 2021a) gave further evidence of CH₄ oxidation within three trunk layers. It was reported that methanotrophs inhabited inside the bark of *Melaleuca quinquenervia* decreased stem CH₄ emissions by 36 ± 5% (Jeffrey et al., 2021a). In our study, the highest potential CH₄ oxidation rate from the bark of *Populus alba* may explain the large proportion of stem CH₄ uptake at 45, 130 and 200 cm height we observed in the field (Chapter Four). In addition, the lower internal stem CH₄ concentration of *Populus alba* at our study site (discussed below) suggest that high-affinity methanotrophs may be the dominated methanotrophic communities inside the tree trunk, which can consume CH₄ at atmospheric concentration (~1.8 ppm) (Tate, 2015). Isotopic and molecular analyses are needed to focus on identifying the community of methane oxidising bacteria inhabited within bark, sapwood and heartwood and quantifying the rate of CH₄ oxidation by methanotrophs between trunk layers for further research.

5.5.2 The role of stem lenticel on stem CH₄ production and oxidation

Contrary to our hypothesis, we did not observe any significant effect of lenticel presence, and lenticel presence and trunk layer interaction effect on potential CH₄ production and oxidation rates under anaerobic and aerobic conditions (Table 5.2&3). The black diamond-shaped lenticels on the bark of white poplar exhibited a larger area of the cracks at the bottom (<45 cm) of the tree stand and the area of the cracks decreased within tree height. However, stem height and the interaction effect of stem height and lenticel presence did not show any

significant effect on potential CH₄ production and oxidation (Table 5.2&3). Tree stem lenticels play an important role in the exchange of water and gases between the aerenchyma and the atmosphere (Mcbain et al., 2004). Lenticels are usually produced above the surface through a fissure in the periderm and the development of lenticels normally occurs during the first growing season, which can be affected by tree species and environmental conditions (Kuo-Huang & Hung, 1995; Evert, 2006). In addition, gas exchange over lenticels can also be affected by the degree of lenticel opening, which is determined by the developmental stage of the lenticel, species, season and environment (Langenfield-Heyser, 1997). Compared to tree species in wetland, the role of stem lenticels in upland non-flooding tree species may not be remarkable. Therefore, the microbial communities colonised in bark may not show substantial differences between wood areas with stem lenticel and without stem lenticels, which might explain the lack of lenticel effect on the capacity of potential wood CH₄ production and oxidation in our study. Further studies are required to determine microbial communities of methanogens and methanotrophs inhabited inside the bark with the presence of lenticels in various tree species (wetland vs upland) during both growing and non-growing seasons.

5.5.3 Internal *in situ* CH₄ and CO₂ concentration

The internal *in situ* CH₄ concentration of white poplar at our research site was close to or even lower than the atmospheric CH₄ concentration during the two campaigns. This is consistent with the relatively low tree stem CH₄ fluxes of the same poplar trees when averaging the high-frequency measurements over the period of mid-June to mid-August, which were 0.66 ± 21.63 , 0.29 ± 20.95 and 0.93 ± 19.57 $\mu\text{g m}^{-2}$ stem surface h^{-1} at 45, 130 and 200 cm above the soil, respectively (Chapter four). In contrast, other studies found substantial heartwood CH₄ concentrations of *Populus davidiana* and *Carya cordiformis* in temperate upland forests, with low CH₄ concentration in the soil profile (0-80 or 0-150 cm depth) (Wang et al., 2016; Barba et al., 2021). Heartwood CH₄ concentrations depended on tree species and climate conditions in upland forests (Covey et al., 2012; Wang et al., 2017). In addition, CH₄ concentrations in the heartwood showed a nonlinear relationship with heartwood water content and substantial CH₄ production was released when water content reached the threshold of c. 45% in temperate upland forests (Wang et al., 2017, 2021). Although the heartwood water content of all our heartwood samples was $49.0\% \pm 0.01$ at our research site, we did not observe

substantial CH₄ production. It was estimated that approximately 87.7% of living trees emitted less than 1000 ppm CH₄ from heartwood in temperate upland forests, which mainly depended on tree species and soil moisture (Wang et al., 2021). The *in situ* measurements of internal CH₄ concentration are needed from tree scale to global scale in the future.

In contrast to heartwood *in situ* CH₄ concentration, we observed high CO₂ concentration inside the heartwood, which reached 1-2 orders of magnitude higher than the atmospheric CO₂ concentration (Fig. 5.4b, 5.5b). Similar results were reported by other studies, which indicated the significant barriers of CO₂ diffusing from the internal stem to the atmosphere (Teskey and McGuire, 2002; Steppe et al., 2007; Teskey et al., 2018). The great amount of stem internal CO₂ concentration further supported our hypothesis that stem CO₂ emission was mostly originated from the stem and roots respiration, rather than from the transport of CO₂ in the rhizosphere (Chapter four). However, current knowledge of the locations with resistance to radial CO₂ diffusion in woody tissues is still unclear. Future research is suggested to focus on the physical barriers of CH₄ and CO₂ diffusion from the interior stem to the atmosphere (Barba et al., 2019, 2021).

5.6 Conclusion

In this study, we investigated potential wood CH₄ and CO₂ production under anaerobic conditions and wood CH₄ oxidation and CO₂ production under aerobic conditions at 20°C in the dark. Three trunk layers i.e. bark, sapwood and heartwood of white poplar (*Populus alba*) stems were examined, from areas with or without lenticels at 45, 130 and 200 cm stem height. The results showed that potential wood CH₄ production and oxidation rates were significantly higher in bark than in sapwood and heartwood. However, we did not observe any significant effect of lenticel presence on potential CH₄ production and oxidation via incubation, which might be explained by the lack of differences in the microbial communities colonised in bark between wood cores with lenticels and without lenticels. Internal *in situ* CH₄ concentration was close to or even lower than the atmospheric CH₄ concentration during two campaigns of wood core sampling. The results were consistent with previous high-frequency measurements of poplar stem CH₄ fluxes, in which higher hour-to-hour net CH₄ emission and net CH₄ uptake rates largely offset each other. Wood incubation experiments further confirmed bark, sapwood and heartwood capacity of CH₄ production and oxidation. Further

research is needed to focus on identifying the community of methanogens and methanotrophs inhabited within bark, sapwood and heartwood and quantifying the rate of CH₄ production and oxidation between trunk layers of various tree species by isotopic and molecular analyses.

6. Discussion

6.1 Key questions and limitations of the study

The primary aim of this thesis was to determine CH₄ and CO₂ fluxes from both soils and tree stems in temperate forests on mineral soils in the UK, investigate the potential biotic and abiotic drivers, and identify the underlying mechanism of tree stem CH₄ exchange. Therefore, this thesis tried to answer the three key questions raised and discussed below.

1. Do ectomycorrhizal (ECM) fungi presence and biochar application have an effect on soil CH₄ uptake and soil respiration both in the short-term and in the longer term? And if so, what drivers may explain the effects?

Chapter 2 identified the short-term (1-3 years) and long-term (3-9.5 years) effects of ECM mycelium presence and biochar application on soil net CH₄ uptake and soil respiration during 2012 to 2021 in a temperate forest on a mineral soil. The mesh-collar approach used only selects ingrowth ECM species based on hyphal diameter only, but not all types of ECM species are included (Tedersoo and Smith, 2013). The results showed that the effect of ECM mycelium presence on net soil CH₄ uptake fluctuated between positive and negative several times during the first two years of the measurements, whilst after 8.5-9.5 years cumulative CH₄ uptake was significantly smaller when ECM mycelium was present. Although the concentrations of soil ammonium, nitrate and inorganic nitrogen were only measured three times out of the 20 measurement dates, we did not observe differences in ammonium, nitrate and inorganic nitrogen concentrations between the only soil with no ECM treatment and ECM fungi presence treatment when the ECM mycelium effect on soil CH₄ uptake occurred. The presence of soil fungi can change soil chemical conditions, such as soil pH, the availability of organic carbon, N and P (Smith and Read, 2008), which may consequently have an impact on soil CH₄ uptake. We acknowledge the limited sampling frequency of soil inorganic nitrogen concentrations and other unidentified potential drivers such as soil organic matter. Subke et al. (2018) have found higher net soil CH₄ uptake in ECM mycelium presence treatment than those in the soil only treatment at the same research site during short-term five weeks. Compared to our results, the effect of ECM mycelium presence on soil CH₄ uptake over 8.5 years of measurements was more complicated, which might be explained by the response of

the methanotrophs community to large temporal variation of ECM fungi and the nutrient cycling rates (Courty et al., 2008; Burke et al., 2012).

Similar to soil CH₄ uptake, the effect of ECM mycelium presence on net soil respiration was not consistent. The presence of ECM mycelium exhibited both positive and negative effects on soil respiration during the short-term 1-2 years, but we did not observe a significant ECM mycelium effect on cumulative CO₂ flux over 1-9.5 years. The fluctuation effect of ECM mycelium presence on soil respiration may be due to the large seasonal variation and seasonal abundance and diversity of ECM fungi (Shigyo, Umeki and Hirao, 2019). Compared to the ECM mycelium effect, soil CH₄ uptake and soil respiration exhibited stronger seasonal variations over the years driven by soil temperature and soil moisture.

The addition of biochar (from *Miscanthus* pyrolysed at 450°C, pH of 9.25) did not have a significant effect on soil CH₄ uptake and cumulative CH₄ uptake during short-term and long-term over 1-9.5 years. Compared to soil CH₄ uptake, the effect of biochar addition on soil respiration was not consistent. We often did not observe any effect of biochar addition on soil respiration, except for four times out of the 20 measurement dates, which biochar addition decreased soil respiration. The inhibition of soil respiration may be due to the type of biochar we applied to the forest soils, which may reduce soil microbial biomass and activity and thus decrease soil respiration rates (Nakajima et al., 2007; Pokharel et al., 2018). However, we did not observe any significant effect of biochar addition on cumulative CO₂ flux was observed during the short-term and long-term over 1-9.5 years. There were no significant differences in soil moisture and soil temperature between biochar and non-biochar addition treatments when the effect of biochar addition on soil CO₂ flux occurred in our study. The inconsistent biochar addition effect on soil respiration over the entire study period may be due to the fine sandy soil texture at our research site. The results from a meta-analysis showed that compared to coarse and medium texture, no significant effect of biochar addition on soil CO₂ flux was found in fine texture soils (He et al., 2017).

Considering the impact of ECM mycelium presence on greenhouse gas (GHG) budgets in temperate forests on mineral soils, it may be difficult to estimate the soil CH₄ and CO₂ fluxes accurately during the short-term 1-2 years due to the fluctuating gas fluxes with the presence of ECM mycelium. However, the presence of ECM mycelium significantly reduced soil CH₄ uptake rates during the long-term 8.5-9.5 years and therefore may enhance GHG emissions

from the soils. Although biochar application is considered as an approach for carbon capture and permanent storage (Fawzy et al., 2020), a review paper reported the effect of biochar addition in forest ecosystem was inconsistent on GHG emissions with complicated (negative, positive and no effect) impact on soil CH₄ uptake and soil CO₂ emissions (Li et al., 2018). Based on our results, we did not observe any beneficial management practice of the biochar type we applied to the fine sandy soils to mitigate GHG emissions in temperate upland forest soils.

2. Do tree stem CH₄ and CO₂ fluxes show high temporal and spatial variability in temperate upland forests? And if so, what drivers may explain the large variation?

Chapter 3 determined the variations of tree stem CH₄ and CO₂ fluxes within English oak (*Quercus robur*) and Japanese larch (*Larix kaempferi*) from two sites during a growing season in a temperate upland forest. Due to Covid-19 restrictions, we did not take measurements of tree stem and soil CH₄ and CO₂ fluxes during April to May 2020. Stem CH₄ and CO₂ fluxes from oak and larch trees (n=12 per species) were measured by rigid stem chambers at 45 cm above the soil surface. Around one-third of the tree stem CH₄ and CO₂ fluxes (30.36% of the data for each gas flux) were removed due to leakage issues. However, due to the large spatial and temporal variability of tree stem CH₄ flux (discussed below), the objectives of the study were still addressed in terms of the lack of sampling frequency and the leakage issues of rigid stem chambers. Compared to other studies, which found more consistent stem CH₄ emissions in temperate upland forests (Covey et al., 2012; Pitz and Megonigal, 2017; Warner et al., 2017), surprisingly, both oak and larch tree stems showed both stem CH₄ emission and uptake during each measurement at our research site. We did not observe seasonal variation in tree stem CH₄ flux over the spring and summer periods. The lack of a seasonal pattern of stem CH₄ flux may be due to the manual measurements (manual chamber, monthly frequency), which fail to capture the large temporal variability of tree stem CH₄ flux, even though they are important in addressing the spatial variability of tree stem CH₄ flux (Barba et al., 2019, 2021). To study the diurnal pattern of tree stem CH₄ and CO₂ fluxes, we only took gas flux measurements on three occasions during 9:30-16:30 over one day period. However, we observed large daytime and intra-specific variations in tree stem CH₄ flux of both tree species. Stem CH₄ fluxes exhibited large variations within individual trees of both oak and larch, and switched between net CH₄ consumption and net CH₄ emission during the daytime. Tree stem CH₄ fluxes of both tree species did not show any significant correlations with soil moisture, soil temperature, air

temperature, DBH, initial CH₄ concentration, stem CO₂ flux and soil CH₄ flux during the entire measuring period. The large variations in tree stem CH₄ flux including both CH₄ emissions and uptake, made it difficult to identify the potential drivers of tree stem CH₄ flux. Contrary to stem CH₄ flux, tree stem CO₂ flux of both tree species exhibited a seasonal pattern with larger stem CO₂ flux in summer than in spring, which was positively correlated to soil temperature and air temperature and negatively correlated to soil moisture.

In order to address the high temporal variability in stem CH₄ fluxes, we measured stem CH₄ and CO₂ fluxes at a 1.5-hour frequency over a 2-month summer period at a stem height of 45, 130 and 200 cm of three white poplar (*Populus alba*) trees in a temperate upland woodland on campus. We cannot carry out the fieldwork in a forest in terms of time and practical issues for checking the equipment regularly on a farther field site. Consistent with chapter 3, we found both stem CH₄ oxidation and production each day at each stem location. The average frequency of stem net CH₄ uptake on a daily basis was $50.7\% \pm 1.8$. Whilst the diurnal pattern was not strong, normalised stem CH₄ flux at all three heights was significantly larger after midnight (00:00-04:00) than from dawn until afternoon (04:00-16:00), and compared to evening (20:00-24:00), stem CH₄ flux was significantly smaller in the morning (08:00-12:00). The normalised stem CO₂ flux of all three poplar trees exhibited a clear diurnal pattern, with larger CO₂ flux during the night-time (20:00-04:00) than the rest of the day, and smallest CO₂ flux during the morning (08:00-12:00). We did not observe any significant correlations between normalised stem CH₄ flux and soil CH₄ flux, soil moisture and initial CH₄ concentration at the three different heights, and soils mostly exhibited a net CH₄ sink. However, other unidentified potential drivers such as sap flow rate, stem temperature and wood anatomy were not determined in this study. Soil temperature showed a similar diurnal pattern as tree stem CO₂ flux and positively correlated with all three poplar tree stem CO₂ flux at all three stem heights. However, it was indicated that temperature cannot be the only factor to accurately predict stem CO₂ flux (Steppe et al., 2007). Other abiotic and biotic factors may also affect diurnal stem CO₂ flux, such as sap flow rate, sap pH, transpiration rate, nutrient uptake, and production and turnover of root and root-associated organisms (Aubrey and Teskey, 2021).

Although tree stem CH₄ flux has not been included in the global CH₄ budget yet (Saunois et al. 2020), it has been estimated that tree stem CH₄ emissions might contribute less than 0.4% to

the global total natural and anthropogenic CH₄ sources (Wang et al., 2021). However, it is still very challenging to up-scale and model tree stem CH₄ dynamics on a global scale, because of the variance of stem CH₄ exchange in different local and regional upland forests (Pitz and Megonigal, 2017; Wang et al., 2021). Although the drivers of the large hour-to-hour variation in poplar stem CH₄ fluxes are still unclear, the mean tree stem CH₄ flux over two months at our research site was relatively small, as higher hour-to-hour net CH₄ emission and net CH₄ uptake rates largely offset each other. Based on our results, the overall impact of the large within-day variations in stem CH₄ and CO₂ fluxes play an important role in estimating seasonal stem CH₄ and CO₂ fluxes and their overall contribution to the forest GHG budget. In order to address the high temporal and spatial variability of stem CH₄ fluxes, our results indicate the need for both automated high-frequency (hourly frequency) and manual measurements (2-4 weeks frequency) of tree stem gas fluxes to quantify their contribution to overall GHG budgets in upland forests.

3. What is the underlying mechanism of tree stem CH₄ exchange in temperate upland forests?

Measuring stem CH₄ fluxes at different stem heights and in the soil can help elucidate the underlying mechanisms of stem CH₄ flux. In chapter 3, due to Covid-19 restrictions, the study of stem height effect on tree stem CH₄ and CO₂ fluxes was only tested once on oak and larch trees in August 2020. Stem height (45 and 130 cm above the soil surface) did not show any significant effect on stem CH₄ flux of English oak and Japanese larch trees. In addition, we did not find a significant correlation between oak and larch tree stem CH₄ flux and soil CH₄ flux at our research sites, and soils were a net CH₄ sink at all times. Consistent with chapter 3, in chapter 4, there was no height effect (45, 130 and 200 cm above the soil surface) on white poplar tree stem CH₄ flux over the experimental period (60 days). Furthermore, we did not find any significant correlations between CH₄ flux in three poplar tree stems at each height and soil CH₄ flux at our research site, and soils almost always were a net CH₄ sink during the whole period and no potential CH₄ production was found in the 0-50 cm soil profile. In contrast, soil is a net CH₄ source and tree stem CH₄ emissions declined with increasing stem height, suggesting tree stem CH₄ source from deeper layers of anaerobic soils in wetland forests (Pangala et al., 2017; Jeffrey et al., 2019; Jeffrey et al., 2021b). Compared to wetland

forests, the results of our study suggested that the origin of tree stem CH₄ production in temperate upland forests is most likely to be inside the tree stem itself.

In chapter 5, lab incubation experiments were performed to determine the potential CH₄ production and oxidation rates in the three main layers of trunks (bark, sapwood and heartwood) of white poplar (*Populus alba*) trees. The results showed that potential wood CH₄ production and oxidation rates were significantly different between trunk layers, which bark exhibited significantly higher CH₄ production and oxidation rates than those from sapwood and heartwood. In contrast, Wang et al. (2016) reported that the highest potential CH₄ production rates of *Populus davidiana* were observed inside the heartwood rather than bark and sapwood, and the potential bark, sapwood and heartwood CH₄ oxidation rates of *Populus davidiana* were undetectable. However, recent studies discovered methanotrophs from bark, sapwood and heartwood of tree species in subtropical forests (Jeffrey et al., 2021a; Feng et al., 2022) provide further evidence of CH₄ oxidation inside tree stems. Wood incubation experiments confirmed the capacity of CH₄ production and oxidation from bark, sapwood and heartwood, which further suggested that biologically *in situ* tree stem produced CH₄ is the major source of stem CH₄ emission and stem CH₄ oxidation is mainly occurring in the stem itself in temperate upland forests.

6.2 Future research

In order to understand the effect of ECM fungi on soil CH₄ uptake and soil respiration in a long-term (more than 3 years), future studies are needed to focus on 1) the impact of the temporal relationship between ECM fungi and the methanotrophs community; 2) the temporal variation effect of ectomycorrhizal biomass on soil respiration; 3) the response of ECM fungi to belowground carbon and nitrogen cycling rates and their impact on soil CH₄ uptake; 4) the effect of ECM fungi associated with roots on soil CH₄ uptake and soil respiration in temperate forest ecosystems.

To date, the studies on tree stem CH₄ flux in forests are at a novel and exploring stage. Future studies are required to 1) determine the stem CH₄ and CO₂ fluxes using the high-frequency (hourly frequency), within-day measurements of different tree species in upland forests on a global scale; 2) study the diurnal pattern of stem CH₄ flux by measuring stem water content and sap flow rate and comparing the passive diffusive gas transport and active transpiration-

driven mass flow using isotopic techniques in upland forests; 3) identify the community of methanogens and methanotrophs inhabited in bark, sapwood and heartwood and quantifying the rate of CH₄ production and oxidation between trunk layers of various tree species by isotopic and molecular analyses; 4) accurately up-scale the stem CH₄ flux from a site level to a global level and estimate the contribution of stem CH₄ and CO₂ fluxes to GHG budgets in forests.

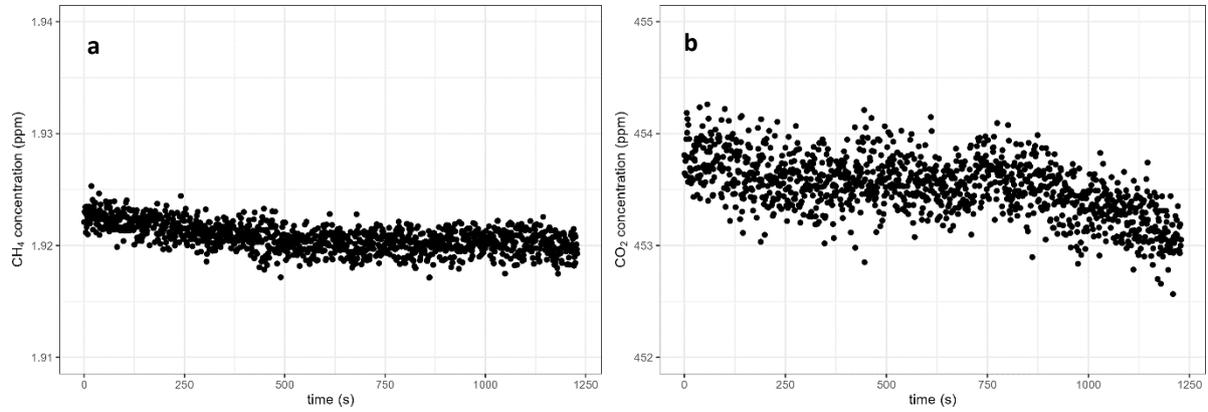
Appendices

Summary:

- **Appendix 1A:** Data of CH₄ and CO₂ concentrations of neoprene foam and non-VOC sealant attached to the rigid tree stem chamber (Chapter 3)
- **Appendix 1B:** Data of tree stem CH₄ and CO₂ concentrations from a removed leakage example and a non-leakage example (Chapter 3)
- **Appendix 2:** Data of wood water content and wood density of bark, sapwood and heartwood from collected wood samples for anaerobic and aerobic incubation (Chapter 5).

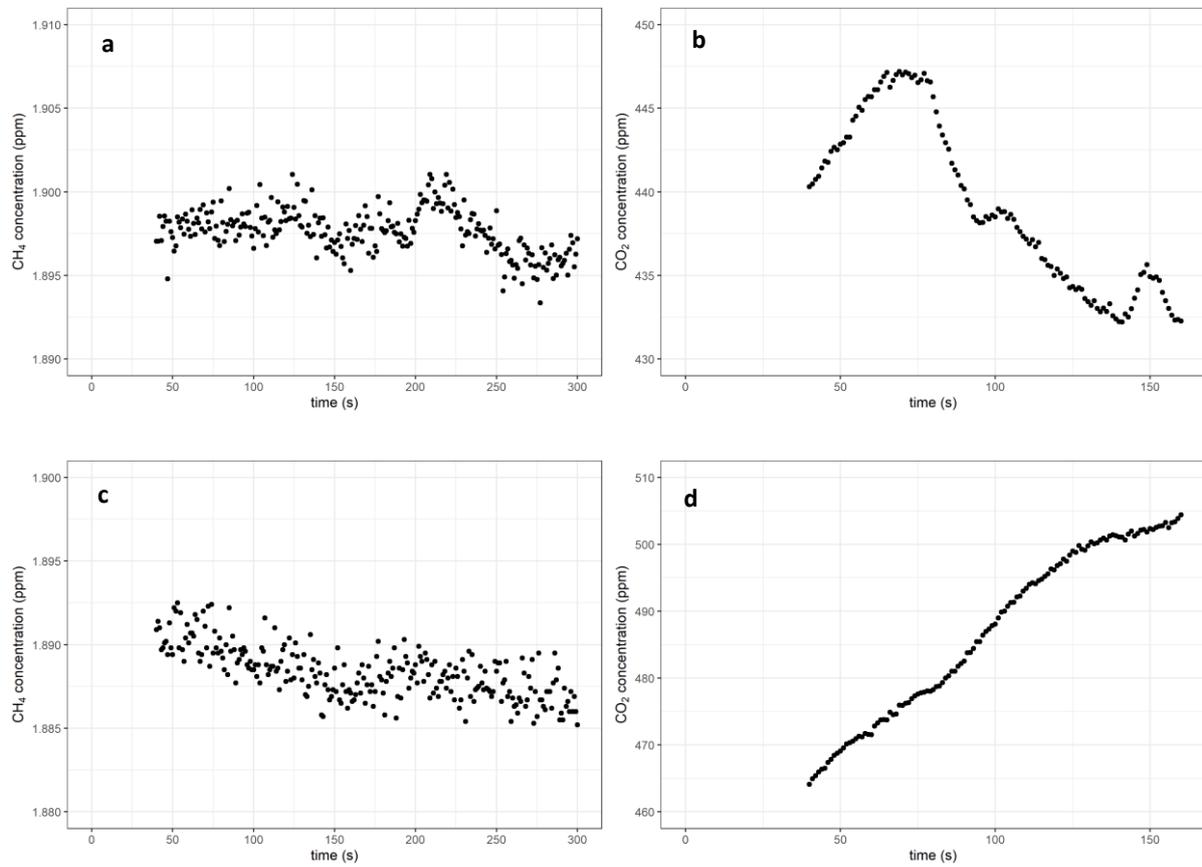
Appendix 1A. Chapter 3

Appendix 1A. Data of CH₄ (a) and CO₂ (b) concentrations of neoprene foam and non-VOC sealant attached to the rigid tree stem chamber.



Appendix 1B. Chapter 3

Appendix 1B. Data of tree stem CH_4 (a, c) and CO_2 (b, d) concentrations from a removed leakage example (a, b) and a non-leakage example (c, d).



Appendix 2. Chapter 5

Appendix 2. Wood water content and wood density of bark, sapwood and heartwood from collected wood samples for anaerobic and aerobic incubation.

Incubation condition	Trunk layer	Wood water content (%) Mean \pm SD	Wood density (g DW cm ⁻³) Mean \pm SD
anaerobic condition	bark	50.01 \pm 0.07	0.41 \pm 0.07
	sapwood	36.52 \pm 0.04	0.47 \pm 0.15
	heartwood	48.62 \pm 0.03	0.45 \pm 0.09
aerobic condition	bark	54.17 \pm 0.12	0.42 \pm 0.07
	sapwood	32.17 \pm 0.07	0.44 \pm 0.02
	heartwood	49.43 \pm 0.04	0.44 \pm 0.03

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