

TROPICAL FOREST
GREENHOUSE GAS
EMISSIONS:

ROOT REGULATION OF SOIL
PROCESSES AND FLUXES

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Abstract

Tropical forested peatlands are a major carbon store and are a significant source of global carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O) emissions. While the role of environmental variables, including temperature and water table depth have been relatively well studied, uncertainty remains in the extent to which plant roots regulate greenhouse gas (GHG) fluxes and peat biogeochemistry. This study examined the role of roots, and root inputs of carbon and oxygen in regulating fluxes from peat under two dominant plant species, *Camposperma panamensis* and *Raphia taedigera*, a broadleaved evergreen tree and canopy palm, in San San Pond Sak wetland, in Bocas del Toro Province, Panama. A combination of *in situ* and *ex situ* experiments were performed between February 2015 and August 2017.

Small scale variation in GHG fluxes and peat biogeochemistry was measured at two distances within the rooting zones of *C. panamensis* and *R. taedigera*. Peat organic matter properties were assessed using Rock-Eval 6 pyrolysis. Results indicated significant variation in CH₄ but not CO₂ fluxes at different distances within the rooting zone, with CH₄ fluxes subsequently linked to measures of the overall size of the available organic carbon pool (S₂). Rock-Eval pyrolysis data was used to construct a three-pool model of organic matter thermostability which indicated significant differences in organic matter composition between peats derived from different botanical origins, in addition to a high level of heterogeneity within the rooting zone.

Changes in GHG production and peat biogeochemical properties in response to the addition of root exudate analogues were assessed in an *ex situ* anoxic incubation experiment. A combination of organic acids and sugars, identified as common forest plant root exudate components, were added over a two week period to peats derived from *C. panamensis* and *R. taedigera*. GHG fluxes varied significantly between treatments but not by peat botanical origin, and were associated with significant changes in soil properties including, pH and redox potential, thereby demonstrating a link between plant root carbon inputs, peat properties and GHG fluxes.

In situ mesocosms were used to assess the effects of root exclusion on peat biogeochemistry and GHG fluxes. Partial and full root exclusion significantly reduced dissolved oxygen concentrations and was associated with greater root necromass. Full root exclusion increased CH₄ fluxes five-six fold compared to partial root exclusion, equivalent to an 86 – 90% reduction in CH₄ oxidation, demonstrating the important role of root inputs of oxygen in mitigating CH₄ efflux from tropical peat.

A ¹³CO₂ pulse labelling experiment was conducted using both *R. taedigera*, *C. panamensis*, and *Symphonia globulifera*, a second broadleaved evergreen tree species, to demonstrate a direct link between plant photosynthesis and CH₄ fluxes, and identify aspects of the bacterial and fungal community associated with the turnover of labile carbon. The extent of ¹³C enrichment of CH₄ differed significantly between plant types (palms vs broadleaved evergreen trees), as did the extent of net CH₄ efflux. Phospholipid fatty acid (PLFA)

biomarker analysis indicated both peat types were dominated by Gram negative bacteria. There was strong ^{13}C enrichment of Gram negative bacteria, supporting their previously proposed role as important decomposers of labile carbon.

Collectively, these results demonstrate that root inputs of carbon and oxygen can strongly regulate tropical peatland GHG fluxes, and that the extent of regulation can vary significantly between tropical wetland plant species from contrasting dominant plant types. This is particularly important in understanding regulatory processes in a globally significant carbon store and understanding possible consequences of land use change in the tropics.

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Chapter 1

Carbon storage and the regulation of greenhouse gas fluxes in tropical peatlands

1.1. Introduction

Peatlands are carbon-rich ecosystems formed when organic matter accumulates under specific environmental conditions which reduce rates of decomposition. As a consequence, peatlands are a critical component of the carbon cycle. Tropical peatlands are particularly important as, despite accounting for only 11% of peatlands by area, they contain between 15 – 19% of total peat carbon (C) (Page et al., 2011, Dargie et al., 2017). Compared to temperate and boreal peatlands in the northern hemisphere, tropical peatlands are relatively understudied, often due to their remoteness. For example, the largest tropical peatland to date was only reported in the literature as recently as 2017 (Dargie et al., 2017).

Tropical peatlands are also a major source of carbon dioxide (CO₂) and methane (CH₄), but the processes by which fluxes are regulated remain to be clarified (Melton et al., 2013). Despite their importance to the global carbon cycle, tropical peatlands are under significant threat from deforestation, land use and climate change. Thus, there is a critical need to understand the processes by which differences in vegetation can regulate greenhouse gas emission (GHG) pathways.

This review assesses the current literature on peatlands globally, but with a particular emphasis on tropical systems, and includes a discussion of geographic extent, carbon storage, decomposition processes, and biotic and abiotic regulation of GHG fluxes.

1.2. Carbon storage in peat ecosystems

1.2.1. Peatland distribution

Peatlands have a global distribution and cover approximately 3% of the Earth's terrestrial surface (Limpens et al., 2008), are a major carbon store, and are a significant source of GHG emissions including both CO₂ and CH₄ (Maltby and Immirzi, 1993). Estimates of global peatland carbon storage range from 330 Tg C (Limpens et al., 2008), to over 600 Tg C (Yu et al., 2010), although significant uncertainties remain in the extent, depth and total carbon storage within sites, particularly within the tropics (Hooijer et al., 2012).

Assessments of peatland distribution have a long history due to the importance of peat as a fuel resource, but more recent assessments have been made in the context of peat carbon sequestration and storage potential. Estimates of global peatland area range from 3.88×10^6 km² (Maltby and Immirzi, 1993) to 4.16×10^6 km² (Limpens et al., 2008). The recent discovery of a large peatland complex in central Africa estimated between 1.31×10^5 – 1.56×10^5 km² highlights the large uncertainties remaining in estimates of global peatland area (Dargie et al., 2017).

Peatlands are broadly classified by latitude, with the majority of peatlands found in the temperate and boreal regions in the northern hemisphere (45 – 60°N). Collectively these peatlands account for $3 \times 10^6 \text{ km}^2$, equivalent to 89% of peatlands by area (Aselmann and Crutzen, 1989, Page et al., 2011). Estimates of tropical peatland area are more uncertain, ranging from 3.34×10^5 – $4.97 \times 10^5 \text{ km}^2$ (Maltby and Immirzi, 1993) to 3.87×10^5 – $6.57 \times 10^5 \text{ km}^2$, with storage estimates of 82 – 92 Gt C (Couwenberg et al., 2009, Page et al., 2011) and more recently 69.6 – 129.8 Gt C (Dargie et al., 2017). South-east Asian peatlands are the largest carbon store, containing approximately 68.5 Gt C, (Page et al., 2011). Central America and the Caribbean contain 5% of global peatland area ($2.34 \times 10^5 \text{ km}^2$) (Page et al., 2011), with Panama containing approximately $7.87 \times 10^3 \text{ km}^2$, equivalent to 1 – 2% of tropical peatlands (Bord na Mona, 1984).

1.2.2. Peat formation, depth and carbon accumulation

Peat is formed by the gradual accumulation of partially decayed plant derived organic matter, driven by generally low rates of degradation arising from distinctly acidic and anoxic environments (Moore, 1989). Over prolonged periods of time, significant depths of peat can amass, particularly when ecosystem net primary productivity (NPP) is high. Accumulation rates are highly variable spatially and temporally, and are determined by a range of environmental and biological variables. In general, greater rates of accumulation are observed within the tropics due to increased temperatures enhancing plant production and peat inputs, in combination with low rates of decomposition (Chimner and Ewel, 2005).

In temperate and boreal peatlands, where the majority of models for carbon accumulation and peat decomposition have been developed, aboveground biomass is believed to be the principle source of material for peat formation, with *Sphagnum* mosses contributing significantly to formation (Gorham, 1991). In contrast, tropical peatlands are frequently forested and peat is therefore composed of a combination of stem, leaf litter and root material (Page et al., 1999, Hoyos-Santillan et al., 2015).

A range of studies have assessed depth and carbon accumulation rates of temperate and boreal peatlands, with significant variation between sites globally, although many estimates of carbon accumulation are made based on a single peat core within a site and therefore do not account for significant spatial heterogeneity arising from topographical and hydrological changes and contrasts in organic matter inputs (Turunen et al., 2002). In North America, a peat deposit in Northern Wisconsin, USA, ranged between 0.1 – 5.1 m deep, with a mean regional depth measurement of 2.1 m (Buffam et al., 2010). Extensive peat deposits in North Carolina, USA were formed 4 – 5 ka BP (kilo annum before present) and have a range of depths from 0.3 – 2.4 m, with 70% under 1.2 m deep (Ingram, 1987b). Maximum peat thickness in a limited subset of temperate sites, however, can be comparable to tropical deposits, with a 9.22 m deposit identified on Browns Island in the Sacramento – San Joaquin Delta, USA (Drexler, 2011).

Although present rates of carbon accumulation within temperate and boreal peatlands are generally lower than tropical peatlands, for example $23.5 \text{ g C m}^{-2} \text{ yr}^{-1}$ in Degerö Stormyr, in Northern Sweden (Nilsson et al., 2008) and $21.5 \text{ g C m}^{-2} \text{ yr}^{-1}$ at Mer Bleue peatland in Ottawa, Canada (Roulet et al., 2007), some accumulation rates are considerably higher. For example, measurements of accumulation across 15 freshwater peatlands from Northern Minnesota to southern Florida ranged from $49 - 88 \text{ g C m}^{-2} \text{ yr}^{-1}$ (Craft et al., 2008).

Temperate peatlands in Europe are similarly diverse in depth, reflecting differences in initiation dates, vegetation inputs and climatic conditions: peat from Loch Dee, Scotland, was found to be up to 6 m thick (Ingram, 1987a) while in the Republic of Ireland sites have been found between 1.2 – 7 m deep (Hammond, 1981). European boreal peatland depth measurements include 6.0 – 7.0 m, recorded in southern Finland and 4.2 m in south-eastern Norway (Ohlson and Okland, 1998, Silvola et al., 1996). Measurements of carbon accumulation include $17.2 \text{ g C m}^{-2} \text{ yr}^{-1}$ in West Siberia (Turunen et al., 2001), $18.5 \text{ g C m}^{-2} \text{ yr}^{-1}$ for a Finnish Mire (Turunen et al., 2002), and $23.5 \text{ g C m}^{-2} \text{ yr}^{-1}$ for Degerö Stormyr in Northern Sweden (Nilsson et al., 2008). Across the entire Holocene, northern peatlands have accumulated between 473 – 621 Gt C, with the majority of accumulation occurring between 11 – 9 ka BP, with a mean rate of $18.6 \text{ g C m}^{-2} \text{ yr}^{-1}$ (Turunen et al., 2002).

Initiation dates for tropical peatlands vary widely due to the local climatic conditions that drove their formation: lowland coastal peatlands located in south-east Asia began forming during the Holocene, 4 – 5.5 ka BP following

the cessation of rising sea levels (Anderson and Muller, 1975). Older peatlands were initiated inland and in sub-coastal areas as early as 20 ka BP, during the Late Pleistocene (Anshari et al., 2001), with cores in Central Kalimantan, Indonesia dated to approximately 26 ka BP (Page et al., 2004) and up to 13.7 m deep (Shimada et al., 2001), although most are between 0.5 – 11 m deep (Page et al., 1999, Hope et al., 2005). The majority of undisturbed peatlands are presently accumulating carbon, with the highest rates currently recorded in the tropics and attributable to higher NPP associated with climatic conditions such as enhanced temperature (Page et al., 2006). Globally, tropical peatlands have accumulated 44 – 55 Gt C since the start of the Holocene, with a mean rate of accumulation of $12.8 \text{ g C m}^{-2} \text{ yr}^{-1}$. Rates for the last 2 ka are higher, ranging from 20 – 50 $\text{g C m}^{-2} \text{ yr}^{-1}$ (Yu et al., 2010). Estimates for carbon accumulation in Central Kalimantan range from $31.3 \text{ g C m}^{-2} \text{ yr}^{-1}$ (Dommain et al., 2011), to $86.6 \text{ g C m}^{-2} \text{ yr}^{-1}$ (Page et al., 2004). Dommain et al. (2011) also reported a mean accumulation rate of $77 \text{ g C m}^{-2} \text{ yr}^{-1}$ for coastal Indonesian peatlands. Accumulation rates of up to $300 \text{ g C m}^{-2} \text{ yr}^{-1}$ have been reported for undisturbed wetland sites in the Federated States of Micronesia (Chimner and Ewel, 2005). Of tropical peatlands globally, south-east Asia features the largest tropical peatlands by volume (1359 Gm^3), with total carbon storage of approximately 69 Gt C (Page et al., 2011).

Peatlands in Africa are generally younger than those in south-east Asia, . Cores in Equatorial Africa have a range of initiation dates: for example 14 ka BP for the Rusaka swamp, Burundi (Aucour et al., 1999) and 6 ka BP along the Volta River, Ghana (Ly, 1980). Rates of carbon accumulation were highly variable

within the Rusaka swamp, ranging from 20 – 200 g C m⁻² yr⁻¹, with lower rates coinciding with drier climatic conditions (Aucour et al., 1999). Estimates of depth range from 0.5 – 11 m with one estimate of over 20 m (Markov et al., 1988). Recent studies in the central Congo basin peatland complex gave an estimated depth of 5.9 m (Dargie et al., 2017).

Peatlands in central and south America are even younger; the most well studied peat deposit in central America, the coastal Changuinola deposit in Panama, was initiated approximately 4 ka BP (Phillips et al., 1997). Upland peatlands in the Andes mountains were initiated as recently 3 ka BP, but show significant depth (4 m) indicating a relatively high mean accumulation rate since initiation of 46 g C m⁻² yr⁻¹ (Chimner, 2008). Depth estimates range from 10 m for Belize (MacIntyre, 1995) and up to 12 m for Panama, with a best estimate of 6 m for the latter (Phillips et al., 1997). Sites in Peruvian Amazonia have reported accumulation rates of 39 – 85 g C m⁻² yr⁻¹ (Lähteenoja et al., 2009). Evidence for carbon accumulation rates for the rest of central and south America are largely lacking in the available literature.

1.2.3. The impact of disturbance, land use and climate change

Despite their important function as a carbon store, tropical peatlands in particular are under significant threat from continued human disturbance, particularly agricultural expansion and intensification (Anshari et al., 2010, Hergoualc'h and Verchot, 2014). Disturbance of peatlands is now understood to have significant effects on peat properties: drainage of peatlands has been reported to increase pH, compaction and therefore bulk density, and decrease

C:N ratios (Anshari et al., 2010), alter nitrogen mineralization and nitrification rates (Reiners et al., 1994) and decrease carbon stocks (Tonks et al., 2017). Disturbance by fire has the potential to greatly enhance carbon emissions with estimates of up to 493.6 Mg CO₂ ha⁻¹ (Hergoualc'h and Verchot, 2014). Drainage of wetlands and the subsequent increase in aerobic decomposition of peat has been reported to significantly increase CO₂ emissions in Kalimantan and Sumatra, by 355 – 855 Mg CO₂ ha⁻¹ (Hooijer et al., 2010). Changes to vegetation, such as the conversion of natural forest to oil palm can lead to significant shifts in soil properties (Tonks et al., 2017), as well as alter micro-climates and GHG flux measurements (Pendall et al., 2010). Hooijer et al. (2012) noted that conversion of forest to *Acacia* or Sago plantations enhanced carbon loss by 90 Mg CO₂ ha⁻¹ yr⁻¹ and 109 Mg CO₂ ha⁻¹ yr⁻¹ respectively over a 25 year period.

Climate change is also hypothesised to have a significant impact on peatland carbon dynamics through changes in air and soil temperature, precipitation and increases in atmospheric CO₂ concentrations (Wright et al., 2011). Across central America air temperature is predicted to alter by +2.2 °C by 2075, and +3.3 °C by 2100. Predicted changes in precipitation range from a small increase of 5%, to reductions of up to 48% (Magrin et al., 2014). Climate modelling within the region indicates that Panama in particular will become both warmer and drier. Reduced precipitation is likely to result in lower water tables within ombrotrophic peatlands, reducing the anoxic zone and enhancing the oxidation of CH₄ to CO₂ (Wright et al., 2011). Laboratory incubation experiments have demonstrated that warming and peat drainage enhance CO₂

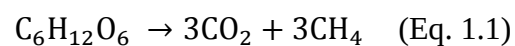
production from surface peat (Sjögersten et al., 2011). Climate change may also have indirect effects on peatlands through changes in plant community composition and through changes to decomposition pathways (Keller et al., 2004).

Changing environmental conditions are therefore likely to have a significant impact on the balance of anaerobic and aerobic decomposition pathways, and rates of peat accumulation. In turn, rates of decomposition determine net efflux of GHGs. It is therefore important to understand both decomposition processes as well as the regulatory role of environmental and biological factors.

1.3. Peat decomposition and greenhouse gas production

1.3.1. Anaerobic decomposition

Anaerobic decomposition ensues when oxygen concentrations are low, and occurs in environments ranging from the ruminant digestive system, to submerged rice fields and peatlands. Approximately two-thirds of naturally produced CH₄ is derived from acetate, and one-third is formed from the reduction of CO₂ (Ferry, 1992). Carbon sources for methanogenesis in peatlands are diverse and include a range of biopolymers such as lignins, cellulose, hemi-celluloses and labile polysaccharides (Conrad, 1999). The mineralisation of organic matter is carried out in successive stages by different populations of microorganisms, but can generally be summarised as:



Initially, biopolymers are hydrolysed in a reaction carried out by aerobes as well as facultative and obligate anaerobes (Le Mer and Roger, 2001). This is followed by the fermentation of monomers into volatile fatty acids, organic acids, alcohols, H_2 and CO_2 by facultative and obligate anaerobes (Segers, 1998). These metabolites are then converted to acetate by syntrophic and homoacetogenic microorganisms. During methanogenesis, the final substrates (primarily acetate, H_2 and CO_2) are processed by strictly anaerobic Archaea under low redox potentials ($Eh < 200$ mV). Other substrates include formate, methylated compounds and alcohols, with different methanogenic species classified on the basis of their preferred substrate (Le Mer and Roger, 2001). Terminal electron acceptors including inorganic nitrate (NO_3^-) and sulphate (SO_4^{2-}), and organic acceptors such as humic acids, are required for the processing of these substrates (Lovley et al., 1996, Lipson et al., 2010). These electron acceptors are used preferentially, driving CO_2 production, before CH_4 production increases through acetate oxidation and reduction (Ye et al., 2012).

1.3.2. Aerobic decomposition

CO_2 fluxes from peatlands can be driven by a combination of autotrophic root respiration and microbial utilisation of carbon substrates in the presence of oxygen (Ueda et al., 2005, Melling and Henson, 2011). CO_2 production rates are generally 2.5 times greater under oxic conditions than under anoxic conditions (Bergman et al., 1999). Aerobic decomposition occurs primarily within the surface layers of the peat which are exposed to elevated oxygen levels from the atmosphere, compared to deeper water-logged and anoxic

conditions. Root release of oxygen may also create small, localised zones with increased redox potential, enhancing the production of CO₂ within the rooting zone either through aerobic decomposition, or the oxidation of CH₄ (Ekschmitt et al., 2008, Hoyos-Santillan et al., 2016a).

1.3.3. Gas transport

GHGs produced through aerobic and anaerobic decomposition can be transported to the peat surface by diffusive, ebullitive or plant mediated pathways. Diffusion, the net movement of molecules from regions of higher concentration to regions of lower concentration, down a concentration gradient is described by Fick's first law (Equation 1.2), where J is the diffusion flux (mass per unit area per unit time), ϕ is the porosity (concentration per unit volume), D is the diffusion coefficient (unit area per unit time), and dC/dz is the concentration gradient (Chanton, 2005). Rates of gas diffusion are therefore regulated by the magnitude of the gradient (which itself may be influenced by prevailing surface climatic conditions such as wind speed) and soil porosity (Clymo and Pearce, 1995). In passing through oxic zones at the soil surface, CH₄ moving by the diffusive pathway can be oxidized to CO₂.

$$J = \frac{\phi D dC}{dz} \quad (\text{Eq. 1.2})$$

Ebullition transports CH₄ directly to the atmosphere and, unlike simple diffusion, partially bypasses the oxic zone, reducing oxygenation. CH₄ is only partially soluble in water (23 – 40 mg L⁻¹ at 0 – 20 °C) (Yamamoto et al., 1976), resulting in the gradual accumulation of pockets of CH₄ within the peat

profile, with recorded volumes of up to 14 L (Chanton, 2005). Bubble release can be triggered through changes in pressure or disturbance and is highly episodic, with a correlation between low air pressure and increased gas bubble volume and bubble frequency (FechnerLevy and Hemond, 1996, Tokida et al., 2005). Bartlett et al. (1990) observed that ebullition occurred in 47% of flux measurements in samples taken within the flooded forest of the Amazon floodplain. Furthermore, the CH₄ released via bubbling contributed the majority of fluxes when it occurred (Bartlett et al., 1990). Conversely, Green et al. (2013) analysed the role of ebullition in temperate wetlands using laboratory incubations from two raised bogs. Net CH₄ fluxes were found to differ between habitats but episodic ebullition contributed less than 3.3% of total CH₄ emissions (Green and Baird, 2013).

The vascular tissue of wetland plants can provide a third important mechanism for gas exchange with the atmosphere which bypasses the oxic surface layer and can thus account for a significant proportion of CH₄ fluxes (see Section 1.5.4), although the precise role of this pathway is still being fully elucidated (Pangala et al., 2017).

The balance of GHG emissions from peatlands, produced by both anaerobic and aerobic decomposition and transported by any of the three pathways, is strongly regulated by a variety of environmental and biological mechanisms.

1.4. Environmental regulation of greenhouse gas fluxes

1.4.1. Hydrology

Water table height within tropical peatlands has previously been reported to correlate with CO₂ and CH₄ emissions primarily by determining whether aerobic or anaerobic decomposition predominates at any given depth. A drop of 10 cm in water table height was found to increase CO₂ emissions by 50% (51.0 mg m⁻² hr⁻¹) and decrease CH₄ emissions by 25% in an Indonesian wetland site (Furukawa, 2005). However, even with drawdown of water tables, deeper peat layers can still be a significant source of CH₄, although this is generally oxidised before it reaches the atmosphere (Inubushi, 1998). A meta-analysis of CO₂ and CH₄ emissions from south-east Asian wetlands noted that when the water table was more than 40 cm below the surface, there were either no net CH₄ emissions or low uptake of CH₄ by the peat (Couwenberg et al., 2009). With water table drawdown of 60 cm, uptake of CH₄ has been measured at 0.1 mg m⁻² hr⁻¹ (Jauhiainen, 2005), with similar patterns observed in other tropical peatlands (Wright et al., 2013a).

Water table height can also display distinct seasonality due to changes in precipitation. For example in the Changuinola peatland in Panama, CO₂ emissions have been found to increase during periods of lower rainfall (Wright et al., 2013a), with a similar effect observed in south-east Asian peatlands (Jauhiainen, 2005, Hirano et al., 2009). Increased CO₂ emissions have also been reported during periods of intense rainfall and high water table level in Changuinola, possibly due to increased litterfall and thus increases in the availability of carbon substrates (Wright et al., 2013a, Wright et al., 2013b).

Weaker relationships, however, were observed between CH₄ flux and rainfall possibly because the majority of the peatland is permanently inundated (Wright et al., 2013b). More pronounced seasonal variation in CH₄ fluxes have, however, been reported in tropical peatland sites in eastern Panama (Walter and Heimann, 2000).

1.4.2. Temperature

Increases in temperature correspond to enhanced rates of decomposition and gas production, particularly within temperate and boreal peatlands where there is greater fluctuation in temperatures. MacDonald et al. (1998) noted the strong influence of temperature on CH₄ flux in field experiments in a blanket bog in Northern Scotland, with emission increasing by 11.4 $\mu\text{mol m}^{-2} \text{hr}^{-1} \text{ }^{\circ}\text{C}^{-1}$ from 5 – 30 $^{\circ}\text{C}$ (Macdonald et al., 1998). The higher temperatures recorded within tropical peatlands can contribute to enhanced rates of decomposition (Chimner, 2004) but with temperatures consistently high relative to temperate peatlands (> 20 $^{\circ}\text{C}$), hydrology and vegetation inputs are more significant in contributing to differences in GHG flux between sites (Jauhiainen, 2005). Laboratory incubations using samples from subtropical peatlands in Sumatra have indicated that CO₂ emissions rates can double with a 10 $^{\circ}\text{C}$ increase in temperature ($Q_{10} = 2$) (Brady, 1997). Diurnal variation in CO₂ fluxes has also been reported, with decreases in efflux overnight possibly driven by reductions in decomposition rates from lower temperatures, or declines in root-derived CO₂ (Wright et al., 2013b).

1.4.3. Chemistry

pH and nutrient availability are amongst a range of biogeochemical factors that can determine peat decomposition rates in boreal, temperate and tropical peatlands (Gorham, 1991). Incubation experiments with temperate peats indicate the CH₄ production is highly sensitive to changes in pH, with production increasing at more neutral pH and then subsequently declining at high, alkaline pH (Dunfield et al., 1993a), although differences *in situ* in peatlands frequently show poor correlation between fluxes and pH, possibly due to the limited range of measured pH (Couwenberg et al., 2009). Positive relationships between CO₂ fluxes and dissolved organic carbon (DOC) have previously been observed, likely due to the increased availability of carbon substrate for microbial communities, although other indicators of nutrient availability, such as C:N, have not consistently been reported as significant determinants of GHG fluxes (Couwenberg et al., 2009, Wright et al., 2013b).

Nutrient availability can also indirectly regulate GHG emissions through influencing plant community structure. For example, the Changuinola peatland in Bocas del Toro province, Panama, features a strong nutrient gradient with declining phosphorus, nitrogen and potassium towards the central peat dome. The nutrient gradient is matched by seven successive plant phasic communities which will influence GHG emissions through a range of biological pathways including contrasts in litter inputs and root structures (Sjögersten et al., 2011), and differences in microbial communities (Troxler et al., 2012). Similar links between plant community composition and site nutrient status of wetland ecosystems have widely been reported at other sites in the tropics, including

south-east Asia (Page et al., 1999) and central and south America (Johnson and Rejmankova, 2005, Vegas-Vilarrubia and Laseras, 2008).

1.5. Biological regulation of greenhouse gas fluxes

1.5.1. Vegetation and litter inputs

Changes in land use, particularly vegetation cover, have been well established as altering CO₂ and CH₄ fluxes in tropical peatlands through a variety of pathways (Sommer et al., 2000, Pendall et al., 2010). Plant species differ in their ability to capture, release and store carbon, with a range of traits influencing each process (Conti and Diaz, 2013). Plants found within stressful environments (such as low nutrient, waterlogged peatlands) differ in growth rates, lifespan and nutrient content of plant tissue, below ground biomass, rooting structures and root growth dynamics, and root exudate profiles (De Deyn et al., 2008). Peat microbial communities have frequently been found to differ under contrasting plant types, and are often linked to contrasts in biogeochemical properties (see Section 1.3.5).

Leaf litter in particular represents a significant source of degradable material which can influence the rate of peat decomposition and GHG fluxes. Litter degradation is a crucial process within all ecosystems in allowing the return of nutrients to soils and thereby maintaining soil fertility (Schreeg et al., 2013). A meta-analysis of 70 litter manipulation experiments, measuring physical and chemical responses to litter presence/absence in boreal, temperate and tropical forests, as well as grassland and shrubland ecosystems, demonstrated that tropical and subtropical forests are more sensitive to litter addition/removal, as

these systems are often phosphorus limited and litter is the dominant source in soils featuring high rates of turnover (Xu et al., 2013).

The chemical composition and intrinsic properties of leaves are critical to determining rates of decomposition. In general, the acidic and anoxic conditions of peat swamps reduce the rate of decomposition, allowing the accumulation of peat. For example, litter from *Campnosperma coreaceum*, a plant common in south-east Asia has higher levels of antioxidants, proanthocyanidin and other phenolic compounds, and possesses also features high levels of anti-microbial activity compared to species that typically characterise secondary forest, such as *Pandanus atrocarpus* (Yule and Gomez, 2009).

Concentrations of phenolic compounds are particularly important in organic matter decomposition in both soils and peats and have been proposed as key drivers of both decomposition and nutrient cycling (Min et al., 2015). Within tropical peatlands, abundant phenolic compounds include lignins and tanins (Yule et al., 2016). Phenolics accumulate under anoxic and low pH conditions, due to the inhibition of phenol oxidase and peroxidase enzymes (Freeman et al, 2001, Freeman et al., 2004), with degradation occurring more slowly than other organic matter fractions (Hoyos-Santillan et al., 2016b). Accumulation of phenolic compounds can subsequently result in the inhibition of other hydrolytic enzymes responsible for peat decomposition and providing a possible mechanism, combined with anoxic and acidic conditions, for organic matter accumulation (Freeman et al, 2001). The role of phenolic compound

accumulation has been less well studied in tropical peatland systems compared to temperate or boreal sites. In one study in the Peruvian Amazon, high concentrations of phenolic compounds found in peat collected from peat sites at Madre de Dios were proposed as the most likely inhibitor of net CH₄ flux, supporting their role as inhibitors of decomposition (Winton et al., 2017).

Multiple studies have identified that leaves from different species decay at different rates, with a range of chemical variables (concentration and type of phenolic compounds, N concentration and lignin:N ratio), as well as physical traits as important predictors (Powers et al., 2009). In a decomposition study incorporating sites in Africa, central and south America, the Pacific and south-east Asia, Powers et al. (2009) noted that bay leaves (*Laurus nobilis*) decomposed more slowly than palm fronds (*Raphia farinifera*), despite having higher N content and a lower lignin:N ratio, indicating the importance of both chemical traits and physical traits (e.g. leaf toughness) in determining decomposition rates. Biotic feedback mechanisms have also been noted, with enhanced litterfall increasing fine root proliferation in response to more readily available nutrients (Sayer et al., 2006), as well as increasing litterfall, indicating that additional leaf litter removes a limitation in forest productivity (Sayer and Tanner, 2010a). However, while the organic chemistry of inputs is important in regulating decomposition and GHG fluxes, the relationship can be altered through changes in the environment. For example, under anoxic conditions, CO₂ and CH₄ efflux closely corresponded to lignin concentrations in depth profiles the Changuinola peat deposit in Panama, but the relationship

between CH₄ efflux and peat chemistry became decoupled following aeration (Hoyos-Santillan et al., 2016b)

1.5.2. Belowground biomass and root adaptations

Root biomass and root adaptations are amongst the most influential traits for driving differences in carbon and nutrient cycling and are crucial for plant survival in stressful environments (Canadell et al., 1996). Estimates of belowground biomass are crucial to understanding carbon storage and dynamics within ecosystems, as decaying roots can represent a significant carbon source, alongside leaf litter in forest ecosystems (Cairns et al., 1997, Hoyos-Santillan et al., 2015, Upton et al., 2018). Although root biomass has been identified as accounting for temporal and spatial variability of CH₄ emissions from rice plants (Sass and Fisher, 1997), this effect has not been consistently noted across all studied wetland species and may not apply in forested peatlands. For example, differences in root biomass did not account for differences in CO₂ and CH₄ fluxes from *Raphia taedigera*, *Camposperma panamensis* and *Cyperus* sawgrasses in a tropical peatland (Wright et al., 2011).

Maintaining root oxygenation can be problematic for roots which are frequently subjected to anoxic conditions (Armstrong et al., 1991). Oxygen has a low diffusion coefficient in water, and consequently waterlogged soils are generally anoxic as even low levels of oxygen demand are sufficient to deplete limited supplies (Armstrong et al., 1991). Low oxygen availability can lead to self-poisoning in plant tissues from the accumulation of fermentation end-

products and free radicals, cytoplasmic acidosis and energy starvation both in the roots but also in aboveground plant systems dependent on full root functionality (Vartapetian, 1997, Armstrong et al., 2000). A variety of adaptations exist to limit plant injury due to anoxic conditions and to facilitate the oxygenation of roots in waterlogged soils including structural morphologies such as root diameter and number, the presence of internal structures including aerenchymateous spaces and barriers to radial oxygen loss, and physiological processes including restricting demand for oxygen (McDonald et al., 2002). Within strongly anoxic environments, eventual root size is constrained through the consumption of oxygen by tissues along the length of the aerenchymateous pathway (Visser et al., 2000).

Root adaptations to waterlogged, anoxic conditions include knee roots, adventitious roots, lenticels and aerenchymateous tissue (Vartapetian, 1997, Cronk and Fennessy, 2001). *R. taedigera* has knee roots which rise above the surface of the water table and are able to enhance the aeration of the root mat (Avalos et al., 2005). Adventitious roots present in other wetland species emerge from the original root system or the stem of the plant, rather than as branches of a primary root, often in response to flooding. Functions include compensating for the decomposition of the original root system, increasing the availability of plant growth hormones, and enhancing the oxygen availability in the rhizosphere (Tsukahara and Kozlowski, 1985, Pimenta et al., 1998). On *C. panamensis* stems, lenticels enhance the rate of oxygen diffusion to submerged roots and also allow the egress of accumulated waste products such as ethylene (Pimenta et al., 1998).

Enlarged spaces for gas transport in the primary and secondary root cortex (aerenchyma) are a widespread adaptation in wetland plants (Armstrong et al., 1991). The development of aerenchyma provides a low diffusive resistance internal pathway for the longitudinal transport of gases to submerged tissues in an otherwise anaerobic environment, as well as reducing the oxygen demand per unit volume of root tissue, enhancing the availability to the growing root tip where demand is greater (Armstrong et al., 1991, Colmer, 2003a). Although primarily formed either during the course of normal plant development under normal environmental conditions, high temperatures, drought and nutrient deficiency can also trigger formation (Evans, 2004). *R. taedigera* features an extensive aerenchymateous root system which may allow increased transfer of CH₄ which bypasses the oxic-anoxic interface on the peat surface where significant CH₄ oxidation can occur (Wright et al., 2011).

1.5.3. Root oxygen loss and root carbon inputs

While decomposing roots are a significant component of tropical peatland organic matter, live roots can release substantial quantities of oxygen and carbon into the peat at depth. Wetland plants feature a range of adaptations to ensure root oxygenation, but significant oxygen can be lost by lateral movement through root tissue. Increased oxygen loss can limit root size and hence nutrient uptake. A range of wetland species reduce oxygen loss through the formation of a partial or complete barrier in their epidermis, exodermis or sub-epidermal cell layers. Increased root diameter can also reduce this loss (Visser et al., 2000, McDonald et al., 2002).

In soil profiles, oxygen concentrations generally decrease with depth, with many biological processes limited to the upper aerated horizons for this reason (Ekschmitt et al., 2008). The depth of the aerobic layer is dependent on soil gas diffusivity and the rate of oxygen consumption by aerobic activity (Iiyama et al., 2012). Watson et al. (1997) found that the presence of roots in the peat increased the transport of oxygen through peat profiles by $500 \mu\text{mol O}_2 \text{ m}^{-2} \text{ hr}^{-1}$ to $3200 \mu\text{mol O}_2 \text{ m}^{-2} \text{ hr}^{-1}$. In the absence of roots, aerobic reactions were limited to the upper 3 cm of peat (Watson et al., 1997).

The loss of oxygen from roots can result in highly localised aerobic zones at depths that would otherwise be anoxic, reducing CH_4 emissions either through oxidation or the inhibition of production. Root oxygen loss from *R. taedigera* was found to be a significant regulator in reducing CH_4 fluxes from peat monoliths (Hoyos-Santillan et al., 2016a). Dissolved oxygen concentrations have been found to correlate to fluctuations in the water table, with increased concentrations with lowered water table levels (Estop-Aragones et al., 2012). Anoxic conditions however, can also occur above the water table if consumption exceeds the diffusion rate of oxygen (Iiyama and Osawa, 2010).

In addition to the release of oxygen, plant roots exude a variety of compounds into the rhizosphere which can mediate both positive and negative interactions with microbial communities, parasitic organisms and invertebrates (Badri and Vivanco, 2009). Root exudates serve a variety of functions including as a nutrient source and chemo-attractant for soil microbial communities (Broeckling et al., 2008) and chelators of low availability soil nutrients

(Dakora and Phillips, 2002, Strom et al., 2002). Exuded compounds include carbohydrates, amino acids, organic acids, lignins, proteins and enzymes. Rates of root exudation have been shown to be linked to rates of carbon fixation and are therefore likely to have a strong regulatory role for gas fluxes and microbial communities, particularly in tropical ecosystems with high levels of NPP (Badri and Vivanco, 2009). Strong diurnal patterns in CO₂ and CH₄ fluxes underline the link between root exudation and carbon fixation (Tang et al., 2005, Wright et al., 2013b). Furthermore, the ¹⁴C isotopic signature of CH₄ emissions has been found to be comparable to contemporary values for atmospheric CO₂, supporting a probable link between plant photosynthesis and CH₄ efflux (Chanton et al., 1995).

Transfer of photoassimilates from the leaves to the roots is dependent on the rate of transport within plant vascular tissues. Compounds produced in leaf tissue via photosynthesis are loaded into the phloem where flow is driven by hydrostatic pressure gradients. At the roots, diffusion and active transport are responsible for unloading, with the movement of solutes further decreasing osmotic pressure and maintaining the gradient. Rates of transport vary from 0.2 – 2 m hr⁻¹ but generally are confined between 0.5 to 1 m hr⁻¹ and are partially dependent on changes in xylem water potential (Kuziyakov and Gavrichkova, 2010). In the roots photoassimilates are partitioned for incorporation into new growth, root respiration and the release of root exudates.

Despite being released at relatively low concentrations ($0.1 - 0.3 \text{ mg C g}^{-1} \text{ soil day}^{-1}$), root exudates are generally highly labile and can therefore be rapidly utilised by microbial communities (Kuzakov and Domanski, 2000), resulting in significant CO_2 and CH_4 production (Figure 1.1). The composition of root exudate profiles can be highly variable between species, ecosystems and with plant age and development. For example, root exudate profiles of forest species are generally high in low molecular weight organic acids, which can be present at concentrations twice those of sugars. Commonly identified components include acetate, citrate and oxalate (Smith, 1976, Grayston and Campbell, 1996, Shi et al., 2011).

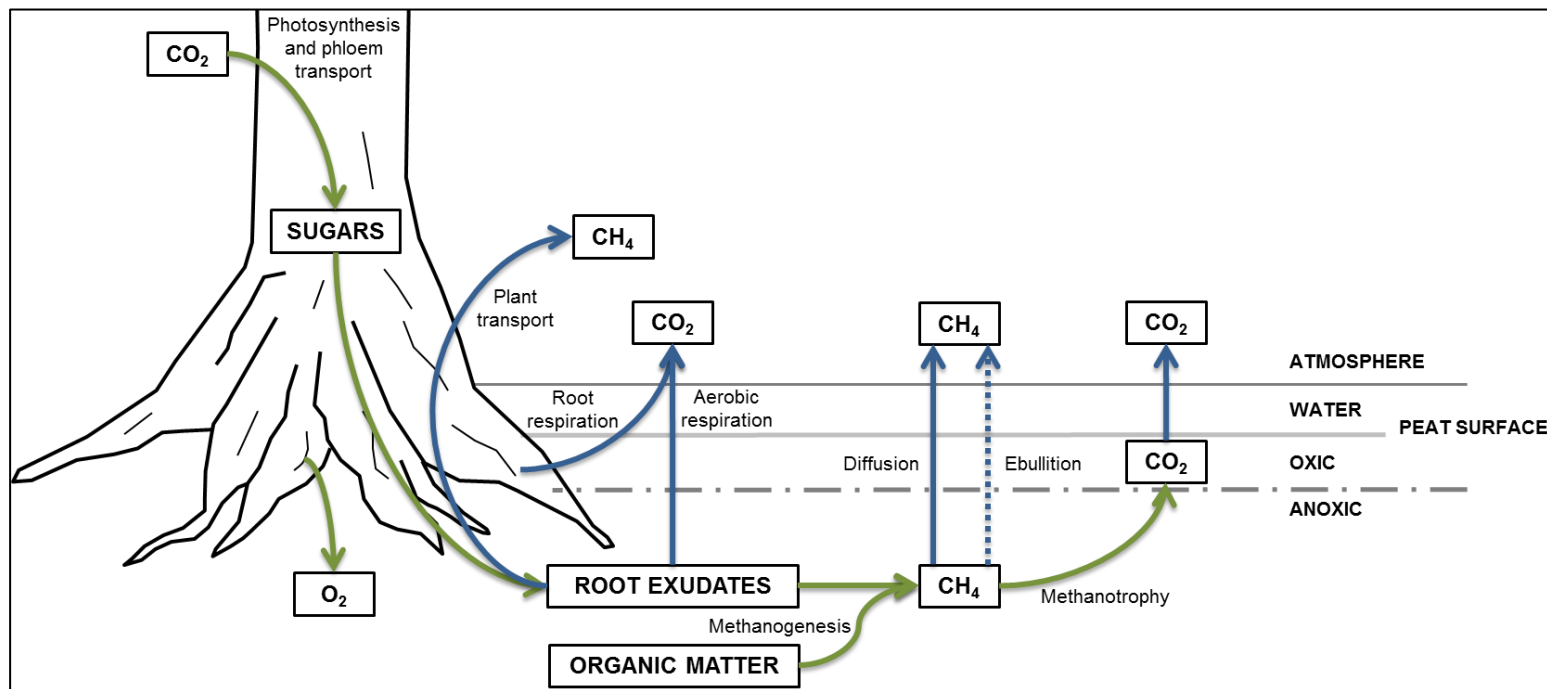


Figure 1.1: Sources and pathways of CO₂ and CH₄ production and transport in tropical peatland plant communities.

Estimates of the relative transfer of photosynthetic products to the roots and into soils vary and are dependent on plant and soil type, environmental conditions, and the rate of plant growth (Trinder et al., 2008, Shi et al., 2011). In general, the fastest growing organ is the most significant carbon store and receives increased allocation of photoassimilates. In crops, approximately 30% of photoassimilates are transferred to soils (Kuzuyakov and Domanski, 2000) but in trees estimates are more variable, ranging from 40 – 48% transfer to roots in walnut trees (Lacointe et al., 1995), 60% in a one year old pine sapling (Norton et al., 1990), and 13.3% (Studer et al., 2014). Root respiration also can contribute directly to CO₂ efflux. Studies in tropical ecosystems assessing the percentage contribution of root-derived CO₂ (both direct root respiration and microbial respiration derived from root inputs) range from 62% in a Malaysian forested peatland (Melling et al., 2013), 66% in a Panamanian peatland (Girkin et al, 2018a), 38% in a lowland tropical forest in Panama (Sayer and Tanner, 2010b), and 24 – 61% in Amazon forest sites (Silver et al., 2005, Metcalfe et al., 2007).

1.5.4. Plant-mediated gas transport

Wetland plant adaptations for low oxygen environments can also provide a pathway for CH₄ produced at depth in anoxic conditions to bypass oxidative surface peat layers and enhance CH₄ emissions (Figure 1.1) (Joabsson et al., 1999). Studies within temperate biomes have identified a correlation between different surface bryophyte communities and CH₄ fluxes. The presence of *Eriophorum vaginatum* in an otherwise *Sphagnum papillosum* dominated peatland increased CH₄ emissions, primarily through increasing the availability

of aerenchymateous tissue for gas exchange (Greenup et al., 2000). CH₄ efflux from temperate tree stems has also been reported, with mean fluxes of 101 µg CH₄ m⁻² hr⁻¹, representing 20% of total ecosystem CH₄ efflux (Gauci et al., 2010). Similarly, tree stems in tropical mangroves in south-east Asia accounted for 62 – 87% of total ecosystem CH₄ flux suggesting purely ground-based studies of emissions may significantly under-report net ecosystem flux (Pangala et al., 2013). Recent measurements of emissions from trees in the Amazon floodplain indicate they may be responsible for up to 15% of global wetland CH₄ emissions (Pangala et al., 2017). The extent of transport through vegetation varies between species due to differences in vascular structures, suggesting that changes to species composition may alter CH₄ emissions through changes in the balance of contrasting transport pathways (Strom et al., 2005).

1.5.5. Microbial communities and stable isotope probing

Soil microbial communities drive decomposition of organic matter, and are important in biogeochemical cycling generally, particularly in increasing the availability of limited nutrients. Moreover, microbial communities are responsible for significant GHG production and consumption. Microbial communities have previously been reported to differ significantly between contrasting land covers (Bossio et al., 2005, Cookson et al., 2007), and during the processes of land conversion (Kumar and Ghoshal, 2017),.

A range of studies of microbial community function have been conducted, including assessments of biomass, respiration rates and enzyme activities.

Approaches including substrate utilisation profiles, and enzyme assays provide detailed information on the role of microbial communities in driving soil processes. Within peatlands, soil microbial enzyme activity is generally greatest at the surface where there are regular inputs from fresh leaf litter and consistent release of root exudates, with activity decreasing with depth (Jackson et al., 2009), possibly accounting for declines in CH₄ fluxes previously found with depth (Wright et al., 2011). The activity of β -glucosidase, amongst other enzymes, has been shown to link directly to the rate of organic matter decomposition in global wetlands, with greater activity generally detected in surface soil layers. Surface layers of peat also have a greater oxygen concentration through surface diffusion and release by roots, with the presence of oxygen increasing the potential for microbial activity (Jackson et al., 2009).

In addition to extensive phylogenetic analyses, microbial community structures have also been assessed through the abundance phospholipid fatty acid (PLFA) biomarkers, membrane spanning lipids found in varying abundances, across fungi and bacteria (Hill et al., 2000). This, and similar approaches can have limitations as biomarkers are generally only able to define microbial communities into broad clades e.g. Gram positive and Gram negative bacteria, with few biomarkers correlated to any specific species. Moreover, production of biomarkers can also vary greatly depending on a range of environmental stresses, including starvation and nutrient deficiency (Frostegard et al., 2011). Previous studies peatlands have identified high abundances of Gram negative

bacteria, particularly Acidobacteria, and relatively low fungi abundance (Jackson et al., 2009, Troxler et al., 2012, Chambers et al., 2016).

Stable isotope labelling has frequently been combined with specific biomarker analysis, particularly PLFA, as a tool for probing both microbial community structure and function (Lu et al., 2004, Pump et al., 2015). Measurements of ^{13}C label incorporation in CO_2 efflux from microbial communities indicates that labile carbon inputs are rapidly utilised (Ostle et al., 2000, Epron et al., 2011, Churchland et al., 2012). Results from these experiments broadly indicate that bacteria are more important in the decomposition of more labile organic molecules, whereas fungi are more important in driving the decomposition of older, more recalcitrant carbon (de Boer et al., 2005). Several studies have indicated that Gram negative bacteria in particular are important in the decomposition of labile carbon (Bird et al., 2011, Koranda et al., 2014). However, ^{13}C labelling in acidic grassland soils has also shown significant incorporation of label in fungal biomarkers, particularly at high rates of carbon input carbon (Waldrop and Firestone, 2004, de Boer et al., 2005). $^{13}\text{CO}_2$ pulse-labelling in a boreal peatland found the highest proportion of a ^{13}C label was incorporated into fungal (18:2 ω 6c), and gram-negative bacteria biomarkers (16:1 ω 7c and 18:1 ω 7c) shortly after labelling (Tavi et al., 2013).

Methanogenic Archaea and methanotrophic bacteria are of particular interest in peatlands due to their respective roles in methanogenesis and CH_4 oxidation. Analysis of the relative incorporation of $^{13}\text{CH}_4$ into microbial DNA revealed that a range of methanotrophs are able to assimilate CH_4 within peat (Morris et al., 2002). PLFA biomarkers for methanotrophs have previously been reported

to include C16:1 ω 5, C16:1 ω 7, C16:1 ω 8c and C16:1 ω 11 for type I methanotrophs and C18:1 ω 7c C18:1 ω 8c for type II methanotrophs (Roslev and Iversen, 1999, Maxfield et al., 2006, Singh et al., 2007, Mills et al., 2013).

PLFA biomarker analysis is unable to identify possible biomarkers for methanogenesis, however, as the process is limited to Archaea which lack ester-linked lipids. Glycerol dialkyl glycerol tetraethers (GDGTs) are another group of membrane spanning lipid reported as biomarkers for both bacteria and Archaea (Dirghangi et al., 2013). Six types of isoprenoid GDGTs have been identified, including GDGT-0 which is known to be produced by methanogenic Archaea, although not exclusively so (Blumenberg et al., 2004, Naeher et al., 2014). Soils also feature nine branched alkyl GDGTs which can be synthesized by anaerobic bacteria. Previous analyses in peatlands has indicated high abundances of GDGT-0 in warm and wet conditions which favour CH₄ production, as well as a range of branched GDGTs (Zheng et al., 2015). Methanogenic Archaea have been also detected using PCR primers designed to target the *mcrA* gene, which codes for part of the methyl-coenzyme M reductase enzyme which is vital to CH₄ production (Juottonen et al., 2006), with abundance correlating significantly with CH₄ production rates in anaerobic digesters (Morris et al., 2014).

1.6. Tropical peatlands in Panama

1.6.1. Changuinola peatland development

Five Panamanian wetland sites have been recognised under the Ramsar Convention, which lists wetlands of international importance. Of these sites, the

most extensively studied is San San Pond Sak, a 164 km² mosaic of freshwater and marine wetlands in Bocas del Toro Province (Ramsar #611). San San Pond Sak includes the 80 km² peat deposit at Changuinola, an ombrotrophic domed peatland. The oldest deposits in the centre are over 8 m deep with accumulation beginning 4 – 5 ka BP (Phillips et al., 1997).

Over time, the vegetation communities at Changuinola have changed significantly. The site is thought to have been initiated as a freshwater palm and hardwood forest swamp behind mangrove forest. Increasing accumulation of recalcitrant peat primarily derived from woody material (predominantly stem and roots) is likely to have driven the formation of the central peat dome, increasing distance to the mineral horizons and reducing nutrient availability (Phillips et al., 1997). The site currently features a strong nutrient gradient with declining phosphorus, nitrogen and potassium towards the interior of the site, accompanied by increases in hydrolytic enzyme activity, indicating strong belowground nutrient limitation (Sjögersten et al., 2011)

1.6.2. Plant communities at Changuinola

Phillips et al. (1997) noted seven distinct plant successional communities across the Changuinola peat deposit. At the coastal edge, is a *Rhizophora mangle* mangrove swamp, followed by a combination of *Rhizophora mangle*, *Laguncularia racemosa* (white mangrove), and *Raphia taedigera* Mart. (a canopy palm) amongst others. This community is followed by monodominant *R. taedigera* palm swamp, a mixed forest stand of *R. taedigera*, and *Camposperma panamensis* Standl., and *Symphonia globulifera*, two

broadleaved evergreen trees. Following the mixed forest is a monodominant stand of *C. panamensis* and a stunted stand of *C. panamensis*. At the centre of the peat dome is a bog-plain predominantly composed of *Myrica mexicana* and *Cyrilla racemiflora* (Phillips et al., 1997, Sjögersten et al., 2011). Nearby sites, such as the less frequently studied Almirante also feature a similar succession of plant communities (Figure 1.2).

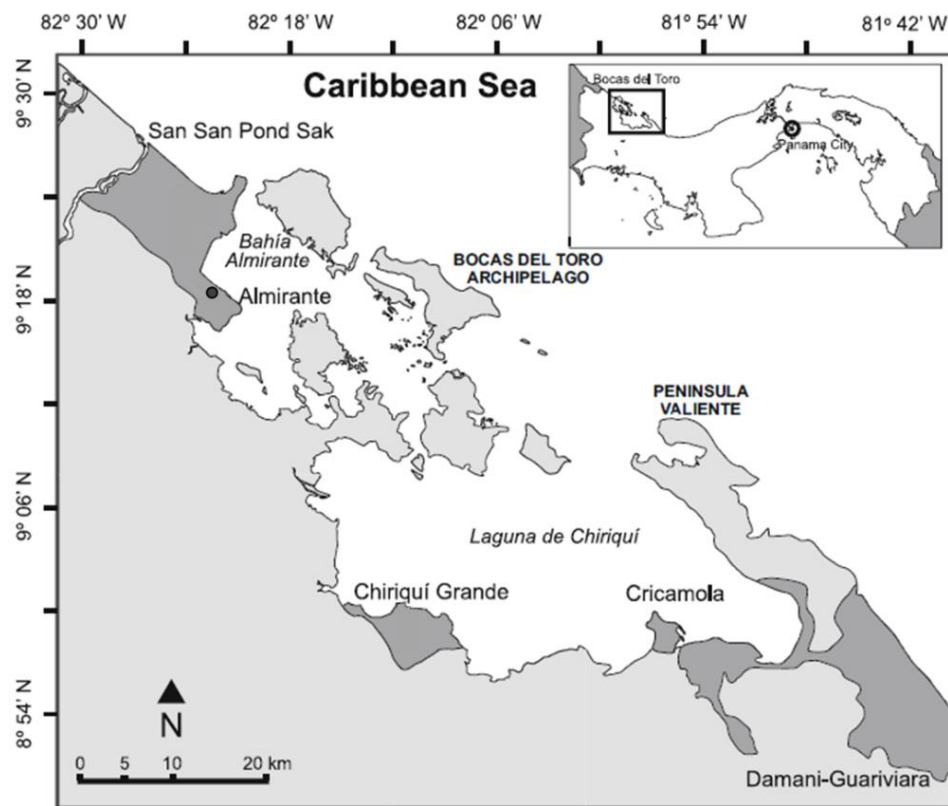


Figure 1.2: Wetlands in Bocas del Toro region of Panama. Sampling was conducted at the site (●) between February 2015 and May 2016.

In this study, samples were collected at a sampling site located in mixed forest stand at Almirante (9°18'31.0"N 82°21'13.8"W) predominantly covered by *C. panamensis* and *R. taedigera*. *C. panamensis* can also grow up to 30 m and

features a long, thin trunk, with buttress roots for support and lenticels to increase aeration of root tissue (Figure 1.3). *R. taedigera* is a large canopy palm with leaves up to 15 m or more, and features an extensive root system with pneumatophores to increase root aeration (Figure 1.4).



Figure 1.3: (a) *C. panamensis* trunk, (b) canopy of *C. panamensis*, and (c) *C. panamensis* root structure.



Figure 1.4: (a) *R. taedigera* palm sapling, (b) canopy of *R. taedigera* fronds, and (c) *R. taedigera* surface root mat.

1.6.3. Climate

Changuinola has a tropical wet climate, with air temperatures that remain fairly constant throughout the year. In the previous 13 years, the mean annual temperature was 26 °C, with mean annual rainfall of 3207 mm. There are two seasons, with the drier season occurring between December and March-April, with generally increased precipitation between April and November. Sampling was conducted predominantly during the dry season and the beginning of the wet season.

1.7. Objectives and hypotheses

While the mechanisms of environmental regulation of CH₄ and CO₂ fluxes have previously been well studied in tropical peatlands in south-east Asia and the Neotropics, the regulatory roles of roots, and root inputs of exudates and oxygen have been less well studied. Previous studies at Changuinola have assessed variation in CO₂ and CH₄ fluxes along vegetation gradients with reference to the impacts of environmental factors (Sjögersten et al., 2011, Wright et al., 2011, Wright et al., 2013a, Wright et al., 2013b), and decomposition processes (Hoyos-Santillan et al., 2015, Hoyos-Santillan et al., 2016a, Hoyos-Santillan et al., 2016b, Hoyos Santillan et al., 2018). Understanding the regulatory role of roots in this ecosystem therefore represents a key knowledge gap for the site, as well as within tropical peatlands globally.

This study directly addresses this knowledge gap and assesses how the processes of root regulation differ between two dominant and contrasting plant species at Changuinola, *C. panamensis* and *R. taedigera*. This will be achieved by assessing the spatial heterogeneity of peat properties within the rooting zones, the response of peats to common root exudate compounds, discerning the role of root oxygen in regulating greenhouse gas emissions, and exploring the link between peat CH₄ fluxes and plant root exudates. The objectives and hypotheses of this study were:

Objective 1: Assess the spatial heterogeneity of peat properties and greenhouse gas fluxes in the rooting zones of two plant species common to tropical peatlands. This objective and associated hypotheses are addressed in Chapter 2: “Vegetation influences on organic matter determine CH₄ fluxes in a tropical peatland.”

- i. Peats collected at different distances from plant stems within the rooting zone will feature distinct biogeochemical properties.
- ii. Carbon emissions will differ between plant species and decrease with distance from their stems
- iii. Differences in carbon emissions between species and with distance from plant stems will be driven by changes in peat organic matter properties.

Objective 2: Assess how composition and quantity of root exudate analogues regulate CO₂ and CH₄ efflux and peat chemical properties. This objective and associated hypotheses are addressed in Chapter 3: “Root exudate analogues accelerate CO₂ and CH₄ production in tropical peat.”

- i. The addition of labile substrates will significantly increase CO₂ and CH₄ fluxes under both species, with more rapid CO₂ changes due to preferential use of terminal electron acceptors
- ii. Increased concentrations of carbon additions will be associated with greater greenhouse gas fluxes
- iii. Differences in CO₂ and CH₄ fluxes between species will be driven by contrasts in peat biogeochemical properties

- iv. The addition of labile substrates will enhance microbial enzyme activities.

Objective 3: Assess how reduced root inputs of carbon and oxygen regulate CO₂, CH₄ and N₂O fluxes and peat decomposition. This objective and associated hypotheses are addressed in Chapter 4: “Roots regulate decomposition and greenhouse gas fluxes in tropical peatlands.”

- i. Root exclusion will significantly reduce concentrations of dissolved oxygen, and plant inputs of oxygen will vary between species.
- ii. Root exclusion will alter organic matter chemistry due to differences in labile carbon inputs and changes in root necromass.
- iii. Changes in organic matter chemistry and root inputs of oxygen will alter the balance of GHG fluxes.

Objective 4: Demonstrate the link between recent photosynthetically fixed carbon and CH₄ fluxes, and assess functional differences in microbial communities between contrasting plant species using ¹³CO₂ pulse labelling. This objective and associated hypotheses are addressed in Chapter 5: “Plant inputs of recent photosynthates drive CH₄ production and reveal microbial community function”

- i. CH₄ enrichment will differ between fluxes from contrasting plant species.
- ii. Microbial community abundance and structure in peats from contrasting botanical origins will differ significantly.
- iii. There will be significant differences in microbial community function in peats derived from contrasting botanical origins

Chapter 2

Vegetation influences on organic matter

determine CH₄ fluxes in a tropical peatland

The work described in this chapter assesses the spatial heterogeneity of peat organic matter properties using Rock-Eval pyrolysis, as well as a range of biogeochemical properties and greenhouse gas (GHG) fluxes *in situ* within the rooting zone of two dominant plant species. In this chapter, I also test links between specific peat properties and peat GHG fluxes.

2.1. Introduction

Plant inputs of leaf and stem material and rhizodeposits are important in GHG emissions from tropical peat (Hoyos-Santillan et al., 2016b). Land use and climate change can significantly alter plant community composition, which may alter peat chemistry through changes in species-specific carbon inputs (Tonks et al., 2017, Girkin et al., 2018b). The consequences of this change are likely to be pronounced as peatlands contain an estimated 15–19% of the global peat carbon stock, and are a significant source of carbon dioxide (CO₂) and methane (CH₄) (Page et al., 2011, Dargie et al., 2017).

Tropical peats derived from different botanical origins have distinct organic matter chemistries which result in different decomposition rates and GHG production (Hoyos-Santillan et al., 2015), particularly in comparison to temperate and boreal peatlands where peat formation is predominantly driven

by graminoid and moss inputs (Turetsky et al., 2014). Under water-logged anoxic conditions that typify peatlands, decomposition is associated with significant CH₄ fluxes. CO₂ is also produced through a combination of autotrophic root respiration, aerobic heterotrophic microbial respiration and the oxidation of CH₄ (Sayer and Tanner, 2010). Differences in vegetation can therefore affect the balance of these emissions through changes in substrate inputs, organic matter composition, microbial communities (Ayres et al., 2009, Keiser et al., 2014), root release of oxygen (Hoyos-Santillan et al., 2016a), and physical properties of the peat (Wright et al., 2013b), and through providing a significant pathway for GHG transport (Pangala et al., 2017).

The extent of plant influences can vary spatially, with rhizosphere soils subject to strong root influences featuring distinct physiochemical properties compared to bulk soils. For example, phosphorus (Hinsinger and Gilkes, 1996) and nitrogen limitation (Landi et al., 2006) are known to drive the release of root exudates, which may in turn be associated with changes in soil properties such as pH (Youssef and Chino, 1989), as well as changes in microbial activity and composition (Gomes et al., 2003, Shi et al., 2011). Combined with differences in biogeochemical properties and microtopography between plant species, peats can display significant small scale (cm to m) variation in chemical properties (Jauhiainen et al., 2005, Wright et al., 2013b). However, the extent to which this variation in peat chemistry under individual plants influences carbon cycling in tropical peatlands is unclear.

Rock-Eval pyrolysis is a method of geochemical screening which was originally used for petroleum exploration but has more recently been applied as a technique to characterise soil and peat organic matter. The technique has previously been used to characterise carbon cycling and organic matter transformation within mangrove (Marchand et al., 2008) and marine sediments (Hare et al., 2014), as well as soil organic matter (OM) dynamics with profile depth (Sebag et al., 2006, Sebag et al., 2016, Upton et al., 2018), and the extent of decomposition and humification in northern peats (Delarue et al., 2013, Biester et al., 2014). During analysis, dried bulk soil samples are thermally degraded in an inert atmosphere at 300 °C producing volatile hydrocarbons. Samples are subsequently pyrolysed through progressing heating from 300 – 600 °C. Emission products (hydrocarbons, CO and CO₂) are quantified by flame ionisation and infrared detectors (Espitalie et al., 1977, Disnar et al., 2003, Newell et al., 2016).

Parameters from Rock-Eval of particular relevance to peat organic matter include the hydrogen index (HI), indicative of atomic H:C, and the oxygen index (OI), indicative of atomic O:C, in addition to TpkS2, the temperature associated with the highest yield of bound hydrocarbons, and S2, a measure of overall hydrocarbons released on the thermal cracking of organic matter for temperatures up to 650 °C. Higher values of HI in peat deposits have been interpreted as indicating the presence of hydrogen-rich labile carbon compounds predominantly found in fresh organic matter inputs (Outridge & Sanei, 2010). HI has previously been reported to decrease with depth, indicating the conversion of aliphatic to aromatic carbon through

decomposition (Schenk et al., 1986). OI generally correlates with the presence of polysaccharides, consistent with the high presence of oxygen particularly in comparison to aromatic compounds. Changes in OI have therefore been proposed as a suitable indicator of polysaccharide decomposition in peat (Delarue et al., 2013).

We used Rock-Eval pyrolysis to assess the heterogeneity of surface peat organic matter properties at two distances within the rooting zones of two plant species commonly found in tropical peatlands, *Camposperma panamensis* (Standl.), a broadleaved evergreen tree, and *Raphia taedigera* (Mart.), a canopy palm. We then linked changes in organic matter properties to GHG fluxes measured over one month. We hypothesised that: i) peats collected at different distances from plant stems within the rooting zone will feature distinct biogeochemical and organic matter properties due to contrasting botanical origins and litter inputs; ii) carbon emissions will differ between plant species and decrease with distance from their stems; iii) differences in carbon emissions under different species and distances from plant stems will be partially driven by contrasts in peat organic matter properties.

2.2. Methods

2.2.1. Study sites

This study was conducted in an 80 km² ombrotrophic peatland at Changuinola, part of the San San Pond Sak freshwater and marine wetland located in Bocas del Toro province, Panama. The peatland was initiated 4000 – 5000 years ago and is 8 m deep at the centre of the peat dome (Phillips et al., 1997). The

peatland supports seven plant communities along a successional gradient from the coast to the central peat dome. The coastal side is dominated by *Rhizophora mangle* mangrove swamp, succeeded by *R. taedigera* dominated palm swamp, a mixed species forest swamp, *C. panamensis* forest swamp, a stunted forest swamp and a *Myrica-Cyrilla* bog-plain (Phillips et al., 1997). The peatland is characterised by a decline in nutrient availability towards the centre of the peat dome (Sjögersten et al., 2011).

Mean annual temperature was 26 °C in the 13 years prior to sampling, with a mean annual rainfall of 3207 mm. During the sampling period in February 2015, peat and mean air were 25 °C and rainfall was 280 mm, which is broadly representative of rainfall throughout the year (Wright et al., 2013b). The water table height varied during sampling but remained consistently just above the peat surface in hollows where sampling occurred, although hummocks were often raised above the water table.

2.2.2. Peat and pore water sampling

Peat samples were collected from a mixed species stand of *C. panamensis* and *R. taedigera* located 600 m from the coast (09° 18' 13.00"N, 82° 21' 13.80"W). Samples were collected from under six *C. panamensis* trees and six *R. taedigera* palms selected to maximise distance to the nearest neighbouring plant (> 4 m) in an area measuring approximately 1 ha. Peat samples were collected at two distances from plant stems, 0.5 m and at 1.5 m, to assess variation in biogeochemical properties within the rooting zone of each individual plant, particularly the effects of reduced root biomass (Figure 2.1).

This assumed that fine root biomass decreased with increasing distance from plant stems (Taskinen et al., 2003, Bauhus and Bartsch, 1996, Mulia and Dupraz, 2006), which has previously been reported in oil palm plantations (Matysek et al., 2017). Samples were not collected at greater distances due to the increased likelihood of influences from other plants including understory vegetation although this was limited, particularly under *R. taedigera* (Wright et al., 2013b). Tropical forested peatlands have previously been characterised as featuring distinct hummocks raised above the water table which are generally raised above the water table throughout the year, and hollows which are periodically inundated depending on changes in the water table. Hummocks have previously been proposed as more significant CO₂ sources than hollows, with hollows more important CH₄ sources during inundation (Jauhiainen et al. 2005; Comeau et al., 2013) although this is not a consistent finding (Hergoualac'h et al. 2017). The microtopography under both *R. taedigera* and *C. panamensis* featured a mix of shallow water pools and raised areas primarily driven by increased the presence of pneumatophores and roots. Sampling of both peat and GHGs was conducted in hollows at both distances to ensure that sampling was conducted in areas continually inundated.

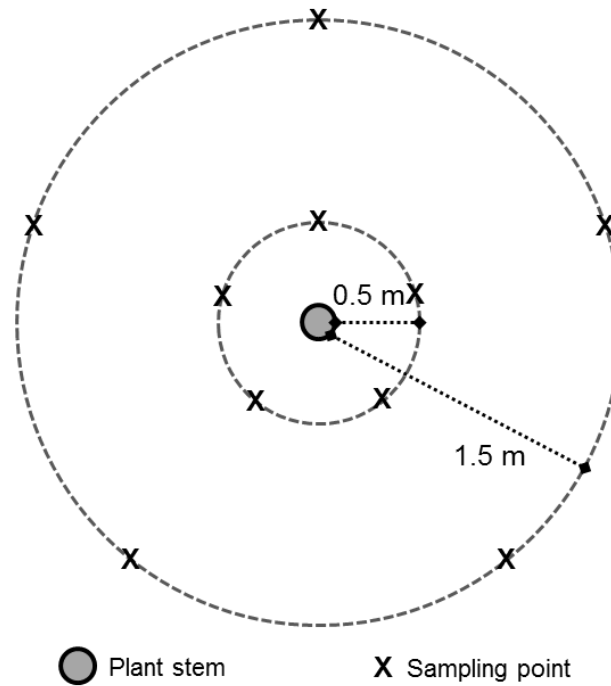


Figure 2.1: Peat sampling strategy at five points around each individual plant stem, replicated across six *C. panamensis* and six *R. taedigera* plants.

Five samples were collected under each plant to a depth of 25 cm using a hand trowel, and combined to create a composite sample. Tree and palm height and diameter-at-breast-height (DBH) were measured for each selected plant, with DBH measured for *R. taedigera* at the highest point before the stem divided into individual palm fronds. A single peat core was collected at both distances under each plant and to a depth of 25 cm to measure fine root density. Cores were weighed and live, fine roots were (< 2 mm) separated by hand based on colour, washed and oven dried at 65 °C for three days. In the absence of any nearby vegetation, it was assumed that live roots were derived solely from the plant at the focal point of sampling. Dry root density was calculated using dry root weight divided by the volume of the core.

2.2.3. *In situ* gas flux measurements

In situ gas fluxes were measured adjacent to each peat sampling site on five occasions over one month from hollows at each site. CH₄ fluxes across nearby sites have previously been shown to be fairly consistent throughout the year between January and June, although CO₂ fluxes are more variable (Wright et al., 2013b). Chambers were placed in hollows, avoiding understory vegetation which may represent an additional organic matter source. Fluxes are therefore a measure of soil respiration rather than ecosystem respiration. Gases were collected from headspaces (0.19 dm³) fitted with a Suba seal[®] rubber septa (Fisherbrand, Loughborough, UK) between 10 am and 4 pm on each sampling day, with samples collected 0, 4 and 8 minutes following chamber installation (Sjögersten et al., 2011). Headspace gases were mixed using a 20 ml syringe and needle and injected into pre-evacuated 12 ml glass exetainers sealed with a screw cap septum, resulting in a slight over-pressurisation (LABCO, UK). CO₂ and CH₄ concentrations were measured using a single injection system with a 1 ml sample loop using N₂ as a carrier gas through a non-polar methyl silicone capillary column (GC-2014; Shimadzu, Milton Keynes, UK). A thermal conductivity detector and flame ionization detector were used to measure CO₂ and CH₄ respectively. Fluxes were calculated using the ideal gas law and assuming the linear accumulation of gases over time (Hogg et al., 1992). Samples that were under-pressurised at the time of analysis were discarded.

2.2.4. Peat and pore water characterization

Peat sub-samples were used to characterize physiochemical properties to 25 cm depth, at 0.5 m and 1.5 m from plant stems. Gravimetric moisture content was determined from the mass lost from 10 g of wet peat, oven dried at 105 °C for 24 h. Peat organic matter content was determined as the mass lost after ignition for 7 hours at 550 °C in a muffle furnace. Bulk density was measured by collecting intact 10 cm × 10 cm × 25 cm sections from the peat surface, oven drying at 105 °C for 24 hours, and weighing. Total peat carbon (C) and total nitrogen (N) content were determined from 0.2 g of dry, homogenised peat combusted using a total element analyser (Flash EA 1112, CE Instruments, Wigan, UK).

Dehydrogenase activity, used as a general indicator of microbial activity, was determined by the reduction of 2,3,5-triphenyltetrazolium chloride (TPC) (Ohlinger, 1995). Two replicate peat samples (1.5 g dry weight) were incubated with 5 ml of 3% TPC at 30 °C for 24 hours, producing triphenyl formazan (TPF). TPF was extracted by shaking with 25 ml of methanol for two hours in the dark. The solution was then filtered in a dark room and the intensity of TPF was measured at 485 nm against a range of known standards. Dehydrogenase activity was expressed in $\mu\text{g TPF g}^{-1} \text{ h}^{-1}$. Previous research has indicated that abiotic reduction of TPC can occur, although this has found to be relatively low compared to other methods for assessing dehydrogenase activity (Oren, 1987). In addition, previous differences in activity have been reported in response to carbon addition (Girkin et al., 2018b), indicating that the contribution of abiotic reduction is likely to be relatively low in these peats.

Peat pore water samples were collected from 25 cm depth using 10 cm long Rhizon samplers made from hydrophilic porous polymer with a pore diameter of 0.1 μm to exclude peat particles (Rhizosphere Research Products, Wageningen, the Netherlands). Samples were frozen during storage and transport. Fulvic acid to humic acid ratios in pore water, indicative of the extent of peat humification and aromaticity, were assessed by spectrophotometric analysis (Grayson and Holden, 2012). Pore water samples were passed through a cellulose filter (Whatman Grade 1, 11 μm) and analysed at 465 and 665 nm (U-2010, Hitachi UV-VIS Spectrophotometer) to calculate the $E_{465}:E_{665}$ index which reflects the ratio of humic to fulvic acids and thus broadly indicates the extent of humification (Uyguner and Bekbolet, 2005). Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were measured using a TOC-V/TN analyser (Shimadzu Corp, Kyoto, Japan).

2.2.5. Rock-Eval 6 pyrolysis

Peat sub-samples were analysed using a Rock-Eval 6 analyser. Freeze-dried powdered peat samples (60 mg) were heated at 300 °C for three minutes before an increase in temperature to 650 °C at a rate of 25 °C per minute in an inert N_2 atmosphere. Residual carbon was subsequently oxidized from 300 °C to 850 °C at a rate of 20 °C per minute. The release of hydrocarbons during the two-stage pyrolysis process was detected by a flame ionisation detector, with an infrared cell detecting the release of CO and CO₂ during the thermal cracking of the OM. Rock-Eval analysis generated a range of standard parameters including S1 (a measure of free hydrocarbons released on heating to 300 °C), S2 (hydrocarbons released on the thermal cracking of organic

matter M for temperatures up to 650 °C), S3CO and S3CO₂ (the CO and CO₂ yielded from the breakdown of kerogen), Tpk2 (the temperature associated with the highest yield of bound hydrocarbons) and total organic carbon (TOC_{RE}). The Hydrogen Index (HI), a measure of hydrocarbons released relative to TOC was calculated as:

$$HI = \frac{S2 \times 100}{TOC_{RE}} \quad (\text{Eq. 3.1})$$

The Oxygen Index (OI), corresponding to the amount of oxygen released as CO and CO₂ relative to TOC_{RE} was calculated as:

$$OI = \frac{S3 \times 100}{TOC_{RE}} \quad (\text{Eq. 3.2})$$

Changes in HI and OI indicate decomposition processes due to dehydrogenation and oxidation of OM, were plotted in a pseudo-Van Krevelen diagram. This plot was originally used to classify coals and subsequently kerogen types but has more recently been applied in the characterisation of organic matter decomposition (Disner et al., 2003).

Additional parameters were derived from the deconvolution of S2 pyrograms using Fityk v0.9.7, an open source curve fitting and data analysis program (Wojdyr, 2010). S2 pyrograms were deconvoluted into six Gaussian signals (F1 – F6) based on maximising R² coefficient values. F1 – F6 values have previously been attributed to organic compounds of increasing complexity and recalcitrance (Disnar et al., 2003, Sebag et al., 2006). F1 signals represent high

labile fresh plant material including simple polysaccharides. F2 signals correspond to lignin and cellulose derived compounds, and F3 and F4 relate to increasingly humified macromolecules. F5 and F6 signals can be attributed to the presence of highly mature and recalcitrant OM, or charcoal. Saenger et al. (2013) proposed combining these signals into three pools representing different phases of carbon thermal stability, with C_l representing highly labile hydrocarbon compounds (F1 and F2), C_i corresponding to the more stabilised carbon pool (F3), and the highly recalcitrant pool, C_p (F4 – F6).

2.2.6. Statistical Analysis

Statistical analyses were conducted in GenStat v17.1. Differences in CO_2 and CH_4 fluxes, peat properties, including Rock-Eval parameters and indices, were assessed using a linear mixed effects model fitted using Residual Maximum Likelihood (REML) to account for variable dependence between sampling plots. Rates of CH_4 and CO_2 production, dehydrogenase activity, and C_l , C_i and C_p were log-transformed to meet assumptions of normality, and tested using a Shapiro-Wilk test. Significance was assessed at $p \leq 0.05$. Relationships between Rock-Eval parameters and mean GHG fluxes (averaged over the sampling period) were assessed using Principal Component Analysis (PCA), based on correlation matrices. Full ANOVA tables are reported in Appendix A.

2.3. Results

2.3.1. Vegetation, peat and pore water properties

C. panamensis trees had a mean height of 15.0 m, and DBH of 41.6 cm, with fine root density ranging from 8.2 – 10.6 mg cm⁻³ across the rooting zone (Table 2.1). Peats were characterised by low bulk density (0.08 – 0.14 g cm⁻³) and consistently high moisture (89.0%). Peats were acidic, and weakly reducing (306 – 311 mV), with low electrical conductivity (115 – 124 µS). Microbial activity in the peats was broadly comparable to previous measurements *ex situ* (3.1 µg TPF g⁻¹ hr⁻¹) (Girkin et al., 2018b). Organic matter content and total carbon was high, but low nitrogen resulted in C:N ratios of 16.8 – 17.7. *C. panamensis* pore water had maximum DOC and TDN content at 0.5 m (166.8 mg L⁻¹ and 22.1 mg L⁻¹). E₄₆₅:E₆₆₅, indicative of the condensation of aromatic carbon, increased slightly at 1.5 m (9.9) compared to 0.5 m (8.5).

R. taedigera palms were smaller than *C. panamensis* trees with a mean height of 8.3 m and significantly lower DBH ($F_{1,10} = 6.27$, $p = 0.03$). Fine root density decreased with distance and was lower than *C. panamensis*, but these differences were not significant. *R. taedigera* peats were characterised by low bulk density (0.06 – 0.10 g cm⁻³) and high moisture content. Peats were acidic, with pH declining from 4.16 at 0.5 m to 3.94 at 1.5, and had low conductivity (70 – 75 µS) and a weakly reducing redox potential. At 0.5 m from the stem, mean dehydrogenase activity was higher than under *C. panamensis* peats (3.6 µg TPF g⁻¹ hr⁻¹) but decreased to 2.9 µg TPF g⁻¹ hr⁻¹ at 1.5 m. Organic matter content was lower at 0.5 m (88.7%) compared to 1.5 m (92.6%), matching the

trend observed under *C. panamensis* but total carbon was broadly similar at both distances (43.6% and 41%). Total nitrogen and C:N both declined at 1.5 m. Pore water collected from under *R. taedigera* at both distances was characterised by high DOC but low TDN content, and consistent $E_{465}:E_{665}$ ratios which indicated humified organic matter. pH declined significantly under both species at 1.5 m from plant stems ($F_{1,10} = 5.16$, $p = 0.049$). Additionally, organic matter content was significantly higher at 1.5 m compared to 0.5 m. ($F_{1,10} = 10.80$, $p = 0.01$).

Table 2.1: Tree characteristics, peat and pore water properties of *C. panamensis* and *R. taedigera*. Means $\pm$ one SE (n = 6).

Species	<i>C. panamensis</i>		<i>R. taedigera</i>	
Distance (m)	0.5	1.5	0.5	1.5
Height (m)	15.0 $\pm$ 0.8	-	8.3 $\pm$ 3.4	-
DBH (cm)	41.6 $\pm$ 2.6	-	30.2 $\pm$ 3.7	-
Fine root density (mg cm ⁻³)	10.6 $\pm$ 2.2	8.2 $\pm$ 1.2	8.14 $\pm$ 1.5	6.47 $\pm$ 1.5
Bulk Density (g cm ⁻³)	0.08 $\pm$ 0.01	0.14 $\pm$ 0.05	0.10 $\pm$ 0.01	0.08 $\pm$ 0.01
Moisture content (%)	89.0 $\pm$ 1.0	89.0 $\pm$ 0.5	89.2 $\pm$ 0.9	90.5 $\pm$ 0.5
pH	3.95 $\pm$ 0.05	3.88 $\pm$ 0.14	4.16 $\pm$ 0.06	3.94 $\pm$ 0.10
Conductivity (μ S)	112 $\pm$ 25	124 $\pm$ 29	70 $\pm$ 12	75 $\pm$ 10
Redox Potential (mV)	311 $\pm$ 9	306 $\pm$ 5	304 $\pm$ 4	306 $\pm$ 5
Dehydrogenase (μ g TPF g ⁻¹ hr ⁻¹)	3.1 $\pm$ 0.2	3.1 $\pm$ 0.3	3.6 $\pm$ 0.3	2.9 $\pm$ 0.3
Total carbon (%)	46.0 $\pm$ 1.5	45.7 $\pm$ 3.4	43.6 $\pm$ 1.7	41.0 $\pm$ 4.0
Total nitrogen (%)	2.8 $\pm$ 0.1	2.6 $\pm$ 0.3	2.6 $\pm$ 0.2	2.5 $\pm$ 0.3
C:N	16.8 $\pm$ 0.71	17.7 $\pm$ 0.9	16.8 $\pm$ 0.4	16.2 $\pm$ 0.4
Organic matter content (%)	94.7 $\pm$ 0.6	96.3 $\pm$ 0.7	88.7 $\pm$ 2.6	92.6 $\pm$ 2.3
DOC (mg L ⁻¹)	166.8 $\pm$ 19.1	149.8 $\pm$ 17.2	184.0 $\pm$ 28.6	188.2 $\pm$ 31.2
TDN (mg L ⁻¹)	22.1 $\pm$ 3.1	21.1 $\pm$ 1.9	21.0 $\pm$ 3.3	22.1 $\pm$ 3.6
E ₄₆₅ :E ₆₆₅	8.5 $\pm$ 0.4	9.9 $\pm$ 0.7	8.7 $\pm$ 1.1	8.9 $\pm$ 0.7

2.3.2. In situ CO₂ and CH₄ fluxes

Mean CO₂ fluxes were consistently lower at 0.5 m under *C. panamensis* (84.50 mg CO₂ m⁻² hr⁻¹), increasing to 108.22 mg CO₂ m⁻² hr⁻¹ at 1.5 m (Figure 2.2a). CO₂ fluxes at 0.5 m were higher under *R. taedigera* (124.50 mg CO₂ m⁻² hr⁻¹), decreasing to 119.92 mg CO₂ m⁻² hr⁻¹ at 1.5 m, although these differences were not significant ($F_{1,37.6} = 0.02$, $p = 0.88$). CO₂ fluxes varied significantly during the sampling period, ranging from 291.98 mg CO₂ m⁻² hr⁻¹ to 32.52 mg CO₂ m⁻² hr⁻¹ ($F_{4,33.8} = 7.32$, $p < 0.001$).

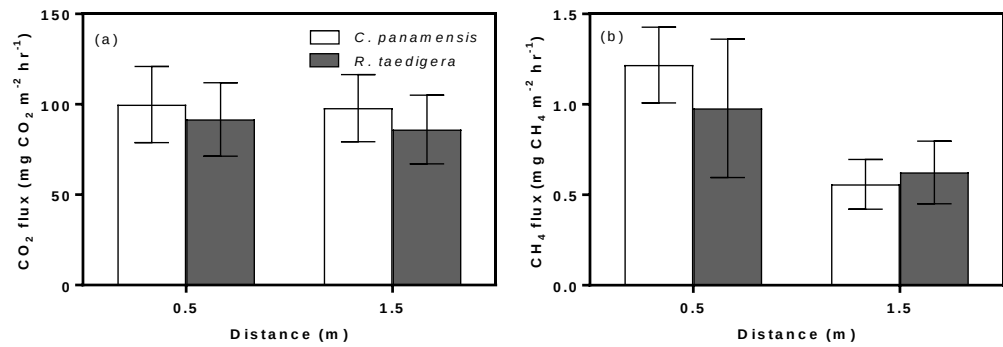


Figure 2.2: (a) CO₂, and (b) CH₄ fluxes at 0.5 m and 1.5 m from *C. panamensis* and *R. taedigera* plant stems. Means $\pm$ one SE ($n = 6$).

In contrast, CH₄ fluxes were significantly higher at 0.5 m compared to 1.5 m under both species ($F_{1,35} = 6.03$, $p = 0.02$). Mean fluxes under *C. panamensis* decreased from 1.25 mg CH₄ m⁻² hr⁻¹ to 0.56 mg CH₄ m⁻² hr⁻¹ (Figure 2.2b), and decreased under *R. taedigera* from 1.00 mg CH₄ m⁻² hr⁻¹ to 0.75 mg CH₄ m⁻² hr⁻¹ but the difference between species was not significant ($F_{1,9.9} = 0.06$, $p = 0.82$). CH₄ fluxes also varied significantly over the course of the sampling period ($F_{4,29.5} = 3.92$, $p = 0.01$).

2.3.3. Bulk organic matter properties

S1, indicative of low weight organic compounds, was low possibly due to depletion by microbial activity. Previously S1 been suggested as being of minimal importance in soils (Disnar et al., 2003) but its importance in peatlands is unclear (Table 2.2). S2 under *C. panamensis* decreased slightly from 123 mg g⁻¹ to 118 mg g⁻¹ at 1.5 m, whereas S3CO and S3CO₂, the CO and CO₂ derived from the breakdown of kerogen, increased slightly. TpkS2, corresponding to the temperature at which hydrocarbon release is maximised, matched the trend in S2 values, decreasing at 1.5 m. The lower TpkS2 at 1.5 m (417 °C) is typical for the thermal breakdown of more labile polysaccharides and lignins, with values over 420 °C indicative of increasingly recalcitrant humic substances (Disnar et al., 2003). TOC was high (40 – 41%) and varied little within the rooting zone. HI decreased from 310 mg HC g⁻¹ TOC_{RE6} to 290 mg HC g⁻¹ TOC_{RE6} at 1.5 m, with a concomitant increase in OI from 200 mg O₂ g⁻¹ TOC_{RE6} to 221 mg O₂ g⁻¹ TOC_{RE6} implying differences in organic matter decomposition within the rooting zone (Figure 2.3).

Table 2.2: Selected Rock-Eval 6 parameters. Means ± one SE (n = 6).

Species Distance (m)	<i>C. panamensis</i>		<i>R. taedigera</i>	
	0.5	1.5	0.5	1.5
S1 (mg g⁻¹)	33 ± 3	31 ± 2	31 ± 2	35 ± 2
S2 (mg g⁻¹)	123 ± 9	118 ± 6	113 ± 8	125 ± 2
S3CO (mg g⁻¹)	19 ± 2	22 ± 1	18 ± 1	17 ± 1
S3CO₂ (mg g⁻¹)	78 ± 6	89 ± 2	701 ± 5	73 ± 5
TpkS2 (°C)	426 ± 2	417 ± 9	414 ± 9	422 ± 2
TOC_{RE} (%)	39 ± 3	41 ± 1	37 ± 2	40 ± 2
HI (mg HC g⁻¹ TOC_{RE6})	310 ± 5	290 ± 14	306 ± 13	316 ± 14
OI (mg O₂ g⁻¹ TOC_{RE6})	200 ± 9	221 ± 11	191 ± 5	185 ± 10

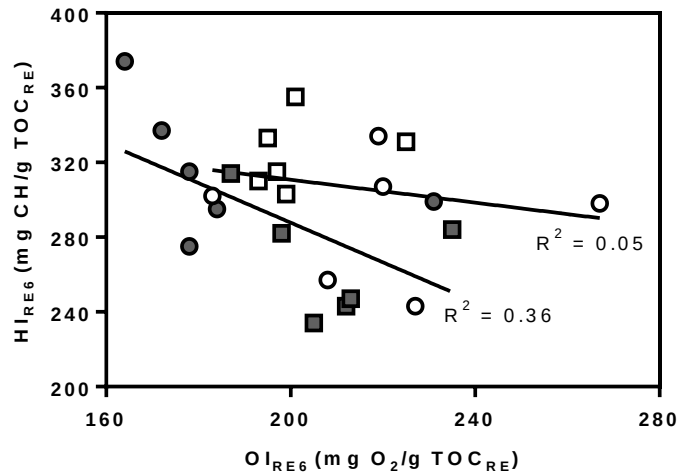


Figure 2.3: Pseudo-Van Krevelen plot linking changes against HI and OI for *C. panamensis* (white) and *R. taedigera* (grey), ■ = 0.5 m, ● = 1.5 m, showing trends in humification (n = 6).

R. taedigera peats showed significant differences in several Rock-Eval parameters compared to *C. panamensis*. In contrast to *C. panamensis*, S2 increased to 125 mg g⁻¹ at 1.5 m from 113 mg g⁻¹ at 0.5 m. There was a significant difference in S3CO ($F_{1,10} = 4.97$, $p = 0.049$) and S3CO₂ ($F_{1,10} = 5.16$, $p = 0.049$) between species, with both higher under *C. panamensis*. Under *R. taedigera*, TpkS2 increased from 414 °C at 0.5 m to 422 °C at 1.5 m, showing the opposite trend to *C. panamensis*. TOC_{RE6} was also somewhat lower, ranging from 37% to 40% but this difference was not significant. In contrast to *C. panamensis* peats, HI increased from 306 mg HC g⁻¹ TOC_{RE6} at 0.5 m to 316 mg HC g⁻¹ TOC_{RE6} at 1.5 m, while OI decreased significantly from 191 mg O₂ g⁻¹ TOC_{RE6} at 0.5 m to 185 mg O₂ g⁻¹ TOC_{RE6} at 1.5 m ($F_{1,10} = 6.37$, $p = 0.03$, Figure 2.3). There was a significant relationship between HI and OI in *R. taedigera* ($F_{1,10} = 5.73$, $p = 0.04$, $R^2 = 0.36$) but not for *C.*

panamensis peats ($F_{1,10} = 0.48$, $p = 0.50$, $R^2 = 0.05$). Concurrent changes in HI and OI are generally indicative of increasing humification of organic matter.

2.3.4. Surface organic matter thermostability

Under both *C. panamensis* and *R. taedigera* and at both distances, the peat labile carbon pool (C_l) was consistently the largest, accounting for between 36% and 42% of the total carbon pool. Moreover C_l exhibited contrasting trends between species (Figure 2.4). There was a significant difference in C_l between species and distance ($F_{1,10} = 6.39$, $p = 0.03$). For *C. panamensis*, C_l was larger at 1.5 m compared to 0.5 m, with the opposite trend observed for *R. taedigera*. The intermediate carbon pool (C_i) varied significantly by species ($F_{1,10} = 16.52$, $p = 0.002$), distance ($F_{1,10} = 8.42$, $p = 0.02$), and in the interaction between species and distance ($F_{1,10} = 8.50$, $p = 0.02$). C_i was higher under *R. taedigera* than *C. panamensis*. Under *C. panamensis*, C_i decreased by 12% at 1.5 m to a minimum of 20%, but under *R. taedigera* it decreased by 2%. The passive pool (C_p) varied significantly with distance, increasing under both species at 1.5 m compared to 0.5 m ($F_{1,10} = 14.28$, $p = 0.004$).

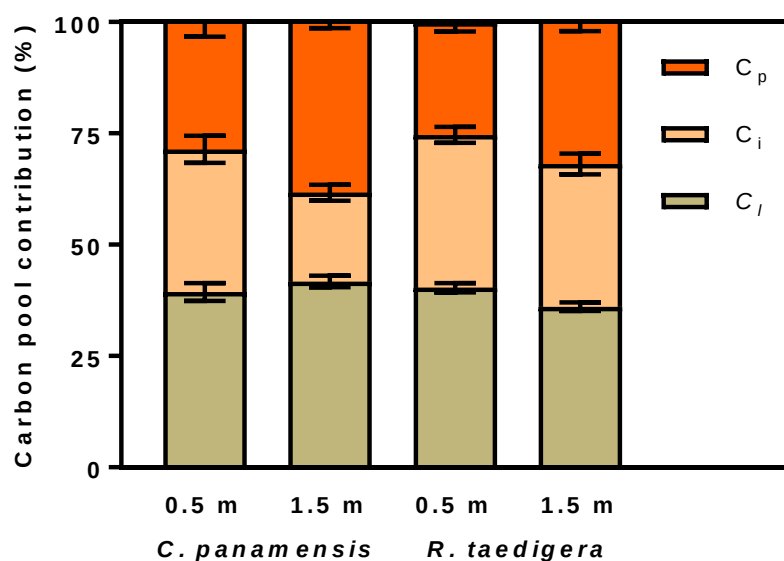


Figure 2.4: Proportions of peat labile (C_l), intermediate (C_i) and passive (C_p) carbon pools for *C. panamensis* and *R. taedigera* in peats 0.5 m and 1.5 m from plant stems. Means $\pm$ one SE (n = 6).

2.3.5. Organic matter properties and CO₂ and CH₄ fluxes

The scores and loadings of the first and second principal components accounted for the majority of the variance (54%) (Figure 2.5). Separation along the first principal component is mainly driven by TOC and TpkS2, and along the second component by HI, C_i , and OI. Peats of both species at each distance display some clustering and demonstrate contrasting trends in peat parameters between species and at each distance. Peat properties are clustered by both species and by distance. *C. panamensis* peats are separated by distance, predominantly by S3CO, S3CO₂, S1 and S2. *R. taedigera* peats are separated more by C_i , HI, S3CO and S3CO₂ but clustering by distance is reduced.

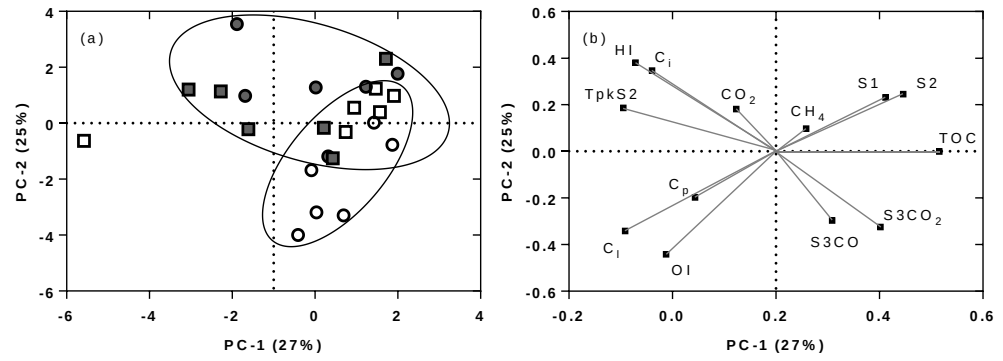


Figure 2.5: (a) Scores for *C. panamensis* (white) and *R. taedigera* (grey). ■ = 0.5 m, ● = 1.5 m (b) loadings from PCA for all species and peats. Combined PC1 and PC2 account for 54% of variance.

Linear regression identified a significant relationship between S2 and mean CH₄ efflux ($F_{1,22} = 9.25$, $p = 0.006$, $R^2 = 26.4$, Figure 2.6), indicating that overall substrate availability, and not a specific carbon pool, was important in driving fluxes. No significant variables were identified for driving CO₂ efflux.

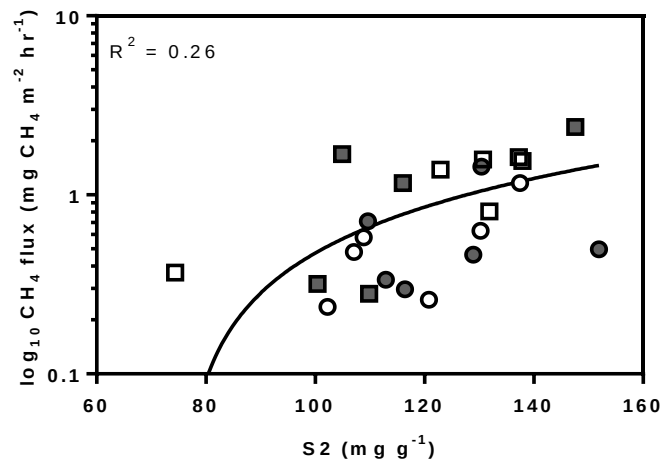


Figure 2.6: Linear regression of S2 and logged mean CH₄ fluxes for *C. panamensis* (white) and *R. taedigera* (grey). ■ = 0.5 m, ● = 1.5 m (n = 6).

2.4. Discussion

2.4.1. Rooting zone trends in biogeochemistry and greenhouse gas fluxes

Changes in fine root biomass with increased distance from plant stems were relatively small and not significant. This may reflect sampling in hollows around plant stems which may feature reduced root biomass compared to hummocks which are formed from roots and fallen leaf litter (Jauhiainen, 2005).

pH and organic matter both declined with distance from plant stems under both species, although these differences were only significant for *R. taedigera*. In general, differences in peat properties between species were reduced compared to previous studies, with only pH differing significantly at 0.5 m. This lack of significant differences is most likely because this study was conducted in a mixed forest rather than monodominant stand which means that peat properties are likely to be influenced to a lesser extent by the previously reported gradient in nutrient availability and microbial communities (Sjögersten et al., 2011, Cheesman et al., 2012, Troxler et al., 2012). Additionally, although individual plants were selected to minimise the presence of nearby understory and canopy vegetation, their influence on soil properties is likely to be limited but cannot be fully discounted.

Aerobic decomposition of organic matter under low water table conditions may drive increases in pH through decarboxylation of organic anions, which involves protonation (Yan et al., 1996). Lower pH with increasing distance from the stems may therefore be associated with reduced decomposition rates,

which is supported by increased organic matter content. Previous studies have indicated that carbon inputs within the rooting zone can substantially increase decomposition, with species specific effects (Kuzyakov and Cheng, 2001, Cheng et al., 2003). Changes in pH have been identified as significant drivers of changes to soil microbial communities (Shi et al., 2011). The lack of significant difference in dehydrogenase activity, used as an overall measure of microbial activity, may be associated with the high organic matter content, as activity is unlikely to be carbon substrate limited.

CO₂ fluxes did not decline with distance under either species, possibly due to limited differences in root density. Declines in root biomass might have been expected to be associated with lower root-derived CO₂. Estimates of root respiration in tropical sites are variable, accounting for 62% of total CO₂ flux from a forested peatland in Malaysia (Melling et al., 2013), but varying from 24–61% in Amazon forests (Silver et al., 2005, Metcalfe et al., 2007). CO₂ emissions can also be driven by microbial utilisation of root exudates and peat OM, and the oxidation of CH₄ (Wright et al., 2013a).

In contrast to CO₂ fluxes, CH₄ varied significantly within the rooting zone under both species, being significantly higher at 0.5 m compared to 1.5 m. Previous work in rice has suggested that labile root exudates are the most important substrate for methanogenic *Archaea* (Lu and Conrad, 2005) and inputs may be greater close to plant stems. Methanogen abundance has previously been found to be higher in the rhizosphere in a wetland soil (Pajares et al., 2016) and, in conjunction with variation in organic matter properties,

may account for the observed fluxes. The smaller differences in CH₄ fluxes between *R. taedigera* may in part be driven by greater inputs of root oxygen which drive the oxidation of CH₄ (Hoyos-Santillan et al., 2016a).

2.4.2. Organic matter properties and thermostability

Deconvolution of the S2 pyrograms into F1 – F6 fractions has previously been used to assess the thermostability of organic matter through changes in the relative proportions of pools of organic polymers (Saenger et al., 2013), although other indices have been calculated based on integration of areas between temperature nodes (Sebag et al., 2016). Previous comparisons of F1 – F6 with FTIR (Delarue et al., 2013, Biester et al., 2014) and ¹³C NMR (Albrecht et al., 2015), indicate only a partial correlation with distinct functional groups as different biomolecules, such as lignin and cellulose, frequently pyrolyse at the same temperature (Sebag et al., 2006). Changes in C_l, C_i and C_p therefore indicate broad scale patterns in thermostability driven by organic matter degradation rather than changes in any one specific functional group (Sebag et al., 2016).

In this study, C_l content under *C. panamensis* and *R. taedigera* was higher than estimates derived through solid state CPMAS ¹³C NMR spectroscopy, which projected that 30% of the carbon pool was labile carbohydrate (Wright et al., 2011), and considerably higher than the 9% estimated for an Indonesian peatland (Orem et al., 1996). High rates of microbial activity may have been expected to rapidly deplete C_l, but it is possible that root exudates and recent root necromass represent a significant carbon input under both species thereby

maintaining a large overall pool. C_i and C_p combined indicate a large pool of humified compounds, most likely derived gradual decomposition of recent litter inputs (Disnar et al., 2003). Overall, deconvolution indicates that rooting zone peats are highly heterogeneous but generally feature a large labile carbon pool, likely derived from fresh litter inputs, in addition to quantities of thermostable biopolymers and humic substances derived from ongoing decomposition processes.

Previous studies have demonstrated that OI and HI are reliable indicators of soil organic matter decomposition (Disnar et al., 2003, Hetenyi et al., 2005, Saenger et al., 2013), with high HI values (over 300 mg HC g⁻¹ TOC) driven by minimally degraded plant tissues rich in polysaccharides, and lower values associated with woody materials with high cellulose and lignin (Marchand et al., 2008). During humification, HI decreases due to increasing aromaticity and dehydrogenation, and OI increases through oxidation, although high values can also be associated with polysaccharides which are relatively rich in oxygen (Hetenyi et al., 2006, Carrie et al., 2012, Sebag et al., 2016). This may explain the low correlation between HI and OI, particularly for *C. panamensis* ($R^2 = 0.05$). In general, high OI and low HI possibly indicates significant humification, particularly under *C. panamensis* peats at 1.5 m.

2.4.3. Small scale variation in organic matter properties

Contrasts in peat organic matter properties with distance from stems between species are likely to reflect the interaction of a range of biotic and abiotic environmental variables. Leaf, stem and shoot litter from *C. panamensis* and *R.*

taedigera litter have previously explained differences in litter decomposition rates within depth profiles (Hoyos-Santillan et al., 2016b). In surface peats *R. taedigera* litter decomposes faster than *C. panamensis* litter, possibly because of higher nitrogen content. However, in waterlogged soils the inhibition of aerobic ligninolytic microorganisms reduces decomposition of lignin rich *R. taedigera* litter (Zeikus, 1981). Differences in plant morphology may also account for some variation in observed organic matter properties. *C. panamensis* has large buttress roots compared to large surface root mats and pneumatophores under *R. taedigera*. *C. panamensis* has small leaves which are likely to represent a more regular carbon input, compared to large palm fronds from *R. taedigera* which will represent a single larger carbon pulse in surface peats (Wright et al., 2013b). Differences in rooting structure can, in turn, strongly influence peat surface microtopography resulting in distinctive raised hummocks and slight depressions (hollows). While the former are often above the water table, hollows can undergo distinct phases of immersion and exposure depending on precipitation (Jauhiainen, 2005). Previously the water table at the site has been reported to change by up to 20 cm (Wright et al., 2013b). In part, the influence of microtopography on fluxes was controlled by sampling only in hummocks which, during the sampling period remained continually inundated, with the water table remaining just above the peat surface.

Vegetation is known to strongly determine microbial communities with both plant species and developmental stage and health known to be important biotic drivers (Berg and Smalla, 2009). Work in temperate and boreal peatlands has

indicated that differences in botanical composition are associated with differences in bacterial communities, with resultant effects on CH₄ cycling (Borga et al., 1994, Robroek et al., 2015), while work in tropical forests has similarly indicated that microbial communities can vary along plant diversity gradients with C:N (Carney and Matson, 2006) and phosphorus availability (Troxler et al., 2012) identified as a key driver of changes in community composition.

In this study the observed contrasting trends in peat organic matter properties with distance under each species are therefore likely to be driven by previously identified contrasts in litter properties, plant morphologies, microtopography, peat biogeochemical properties (particularly pH and C:N and the relative size of carbon pools), and microbial communities.

2.4.4. Linking organic matter properties and greenhouse gas fluxes

CH₄ fluxes were not linked to any individual carbon pool but rather the total size of the relatively labile pool as measured by S2. Generally, surface peat between 0 – 30 cm is the main source of CO₂ and CH₄ but some is produced at significant depth and will therefore be associated with different trends in organic chemistry (Wright et al., 2011).

In contrast to CH₄ production, no significant relationships between CO₂ fluxes and peat properties were identified. This may be because CO₂ can be driven by multiple sources including autotrophic root respiration, microbial respiration of

root exudates, and the oxidation of CH₄, as well as microbial decomposition of organic matter (Kuziyakov and Larionova, 2006).

These results highlight how vegetation influenced organic matter properties can influence CH₄ fluxes by determining substrate availability for methanogenesis. As a consequence, these results show that changes in tropical peatland vegetation in tropical peatlands may be associated with alterations in organic chemistry and hence CH₄ efflux.

2.4.5. Conclusion

Peats derived from *C. panamensis* and *R. taedigera* display strong heterogeneity of biogeochemical properties (pH, total carbon, and organic matter content) within the rooting zone. Changes in the three carbon pools derived from S2 pyrograms, combined with trends in HI and OI highlight how the different litter properties of *C. panamensis* and *R. taedigera* interact with the environment to drive decomposition. The largest pool was consistently found to be C₁, which has previously been linked to highly labile biomolecules. Net CH₄ but not CO₂ fluxes were closely associated to the overall size of the organic matter pool, rather than any one specific group of biomolecules. Collectively, these results demonstrate tropical peats derived from beneath contrasting vegetation types can exhibit significant small scale variation in organic matter properties which can influence GHG emissions. This is the first evidence that changes in dominant vegetation caused by climate or land use change is likely to affect CH₄ effluxes in tropical peatlands if associated with changes in organic carbon pool size. However, it is unclear how specific plant

root exudates, and differences in concentration and composition, regulate both CH₄ and CO₂ production.

Chapter 3

Root exudate analogues accelerate CO₂ and CH₄ production in tropical peat

In the previous chapter, I demonstrated significant heterogeneity of organic matter properties within the rooting zone of two plant species common to tropical peatlands, and demonstrated that CH₄ fluxes are significantly higher closer to plant stems, and are driven by the overall size of the organic carbon pool. The work described in this chapter investigates whether plant root exudate analogues are significant regulators of both CH₄ and CO₂ fluxes from peats derived from contrasting botanical origins.

3.1. Introduction

Peatlands are a globally important carbon (C) store containing up to 610 Gt C due to the gradual accumulation of organic matter through an imbalance in rates of input, degradation and losses from leaching. Tropical peatlands, while only accounting for 11% of total global peatland area, contain up to 88.6 Gt C, equivalent to 14% of total peat carbon, but are threatened by changes in climate and land use (Page et al., 2011). Alongside their role as carbon stores, peatlands are also significant sources of carbon dioxide (CO₂) through plant and soil respiration, and also of methane (CH₄) due to the degradation of

organic matter in anoxic conditions. Indeed, wetland environments (including peatlands) contribute 20 – 39% of global CH₄ emissions and thus play a crucial role in the global carbon cycle (Laanbroek, 2010).

Rates of GHG emissions from tropical peatlands are regulated through the interaction of a range of biotic and abiotic environmental variables including water table height (Chimner and Cooper, 2003, Couwenberg et al., 2009), temperature (Inglett et al., 2011, Hirano et al., 2007), nutrient availability (Sjögersten et al., 2011), oxygen availability (Askaer et al., 2010), and pH (Dunfield et al., 1993b). Vegetation also influences greenhouse gas emissions through its effects on soil properties (Wright et al., 2013b), by providing a mechanism of gas transport (Pangala et al., 2013), and by determining labile carbon inputs in the form of decaying plant material and root exudates (Hoyos-Santillan et al., 2016b).

Plants release significant quantities of photosynthetically derived carbon into the rhizosphere in the form of root exudates, which serve to regulate the surrounding environment. Root exudate functions include acting as a nutrient source and chemo-attractant for soil microbial communities (Broeckling et al., 2008), the detoxification of contaminated soils (Silva et al., 2004), pH regulation (Yan et al., 1996), and chelation of minerals and nutrients (Dakora and Phillips, 2002, Strom et al., 2002). Rates of root exudation are linked to rates of carbon fixation during photosynthesis, and are therefore likely to have

a strong regulatory influence in tropical ecosystems with high levels of net primary productivity (Badri and Vivanco, 2009).

The chemical composition of root exudates varies between species and plant development stage, as well as prevailing environmental conditions, but can include a range of labile sugars, organic acids and amino acids. While root exudates contain low concentrations of carbon compared to other plant inputs, they are utilized rapidly by the microbial community (Kuzyakov and Domanski, 2000). Sugars are the dominant exudate in grassland and agricultural systems but in forest ecosystems, low molecular weight organic acids often occur at two to three times the concentration of sugars (Smith, 1976, Grayston and Campbell, 1996, Shi et al., 2011). Most studies of root exudates have been conducted on agricultural plants such as maize (Baudoin et al., 2003, Henry et al., 2008) or wheat (Kuzyakov and Cheng, 2001), within grasslands (Fu and Cheng, 2002, Bird et al., 2011) or in temperate or boreal forest species (Fox and Comerford, 1990, Shi et al., 2011). Despite the important role of root exudates for soil biogeochemical cycling and the major contribution of tropical forests and peatland ecosystems to the global carbon cycle, few studies have examined the role of exudates within tropical forest or peatland ecosystems (Basiliko et al., 2012).

The objectives of this study were to assess how labile carbon inputs, in the form of root exudate analogues, regulate greenhouse gas production in Neotropical peats, and the extent to which these responses differ between peat derived from two contrasting species that form monodominant stands in

tropical wetlands in the region: the canopy palm, *Raphia taedigera*, and the broadleaved evergreen tree, *Campnosperma panamensis*. We hypothesised that: i) the addition of labile substrates will significantly increase CO₂ and CH₄ fluxes under both species, with more rapid CO₂ changes due to preferential use of terminal electron acceptors; ii) increased concentrations of carbon additions will be associated with greater greenhouse gas fluxes; iii) differences in CO₂ and CH₄ fluxes between species will be driven by contrasts in peat biogeochemical properties, and iv) the addition of labile substrates will enhance microbial enzyme activities.

3.2. Methods

3.2.1. Study sites

Peat samples were collected in February 2015 from San San Pond Sak marine wetland. Samples were collected using a hand trowel from a mixed species stand of *R. taedigera* palm and *C. panamensis* broadleaved evergreen trees, located approximately 600 m inland from the coast (09° 18' 13.00"N, 82° 21' 13.80"W). Differences in vegetation and peat biogeochemical properties have previously been established between the two species (Hoyos-Santillan et al., 2015). Peat samples were collected from under three *C. panamensis* trees and three *R. taedigera* palms at a depth of 10 – 20 cm to reduce the effect of inputs from recent litterfall on the surface horizon. Samples were collected from two points under each tree and combined to give a composite sample. Diameter-at-breast-height (DBH) and tree height were measured for each plant sampled. All peat samples were collected within 0.5 m of the nearest tree, avoiding larger tree roots. Samples were sealed in zip-lock bags and transported to the

Smithsonian Tropical Research Institute station in Bocas del Toro, where samples were refrigerated (2 °C) prior to transportation to the University of Nottingham for analysis. Subsequent references to species refer to peat samples derived from different botanical origins.

3.2.2. Experimental design

In situ studies based on root exudates can be problematic due to the complications of root respiration and the utilisation of other carbon sources by microbial communities. Incubations with root exudate compound (REC) solutions have been applied in a variety of systems to assess the contribution of exudate analogues to changes in microbial communities (Shi et al., 2011) and positive and negative carbon priming effects (Basiliko et al., 2012, Nottingham et al., 2012).

REC treatments were selected using literature data on the most common sugars and organic acids in exudates of 33 tree species (Appendix B). Of these, 11 were classified as sub-tropical or tropical species. Across all ecosystems, fructose, glucose and sucrose were consistently identified as the most abundant sugars in root exudates (Smith, 1976, Jones, 1998, Shi et al., 2011). Amongst tropical species, the most commonly occurring organic acids were acetate, citrate, formate, malate, oxalate and succinate, which were identified in at least half of all species. Dominant RECs were similar between sub-tropical/tropical species, and between temperate and boreal species. No studies were found that identified the RECs of palm species.

Following this analysis, acetate, formate, malate and oxalate were selected using additional criteria. Approximately two-thirds of all CH₄ produced in natural environments has been estimated to originate from methanogenic Archaea using acetate as a substrate (Ferry, 1992). Furthermore, formate (alongside CO₂) contributes to the remaining third of CH₄ production, and has been identified in high concentrations bulk forest soils from the south-east USA (Fox and Comerford, 1990). Malate and oxalate (amongst other organic anions) have previously been shown to enhance plant phosphate uptake (Lopez-Hernandez et al., 1986, Strom et al., 2002), and may therefore have an important role in phosphorus limited peatlands (Sjögersten et al., 2011).

REC solutions were divided into a combination of treatment regimens with addition rates of 0.1, 0.2 and 0.3 mg C g⁻¹ day⁻¹ (calculated using dry weight equivalent). These rates of addition were selected to match typical exudate input rates observed under low, medium and high plant photosynthetic rates and match rates previously used in the literature, and to more accurately assess the effects of exudate profiles which include a range of components (Grayston and Campbell, 1996, Baudoin et al., 2003, Shi et al., 2011, Basiliko et al., 2012). Organic acids and sugars were added in seven combinations consisting of all sugars added together at an equivalent carbon input rate of 0.1 mg C g⁻¹ day⁻¹, all organic acids added together at a rate of 0.2 mg C g⁻¹ day⁻¹, all substrates added in combination at a 2:1 ratio of organic acids to sugars for a total input of 0.3 mg C g⁻¹ day⁻¹ and each organic acid individually added in combination with all three sugars at 0.3 mg C g⁻¹ day⁻¹ (Table 3.1). A 2:1 ratio was selected to match ratios of organic acids to sugars previously reported in

tree root exudate profiles (Smith, 1976). All REC solutions were prepared using the appropriate sodium salt dissolved in DI water and adjusted to pH 5.5 using NaOH. This pH matches field measurements (Table 3.2), and prevents lowering pH on REC solution addition (Renella et al., 2006). Following preparation, REC solutions were sterilised and stored at 4 °C.

Anoxic assays were conducted to simulate the water-saturated and low oxygen conditions found at the site. A peat sample (7.5 g dry weight equivalent) was placed in each of 48 replicate 120 ml glass serum bottles (Kinesis, St. Neots, UK), which were saturated with DI water to give a total occupied volume of 40 ml, leaving 80 ml headspace. Bottles were flushed with nitrogen for two minutes to displace oxygen and create anoxic conditions before sealing with a rubber septa (13 × 19 × 12 mm; Rubber B.V., Hilversum, NL), and an aluminium crimp top. Serum bottles were placed in a 28 °C temperature control room for two weeks to closely replicate *in situ* peat conditions and allow the establishment of the microbial community. Bottles were then opened, flushed with nitrogen to dissipate accumulated headspace gases and then re-sealed for the start of the experiment. REC solutions were added at a rate of 1 ml per day, over 14 days, with 1 ml autoclaved de-ionised water as a control. Headspace samples were collected after seven days incubation (prior to the addition of REC solutions), and on days 15, 22, 30 and 38. Bottles were then opened to allow peat sampling.

Gas samples (5 ml) were extracted from the headspace by syringe and analysed by gas chromatography (GC-2014, Shimadzu UK LTD, Milton Keynes, UK).

CO₂ and CH₄ concentrations were determined using a single injection system, with a 1 ml sample loop that passed the gas sample using N₂ as carrier through a non-polar methyl silicone capillary column (CBP1-W12-100, 0.53 mm I.D., 12 m, 5 mm; Shimadzu UK LTD, Milton Keynes, UK). Thermal conductivity (TCD) and H₂ flame ionization (FID) detectors were used to measure CO₂ and CH₄, respectively (Wright et al. 2011). Gas concentrations were adjusted for incubation temperature (28 °C) and changes in pressure within the serum bottles according to the ideal gas law. Calculations also took account of changes in headspace volume and pressure caused by the addition of 14 ml of REC solutions over two weeks. The potential rate of gas production, expressed as µg CO₂ g⁻¹ or µg CH₄ g⁻¹, was calculated assuming a linear accumulation rate of gases in the headspace (Hogg et al., 1992).

3.2.3. Peat characterization

Sub-samples from each site were used to characterize peat physiochemical properties. Water content was determined by analysis of the mass of water lost from 10 g wet weight peat, oven dried at 105 °C for 24 hours. Organic matter content was determined as the mass lost after ignition for 7 hours at 550 °C. Bulk density was measured by collecting 10 cm × 10 cm × 20 cm sections from the peat surface, and oven drying at 105 °C for 24 hours. Total peat carbon (C) and total nitrogen (N) were determined from 0.2 g dry, homogenised peat combusted using a total element analyser (Thermo Flash EA 1112, CE Instruments, Wigan, UK). Solution pH and redox potential were measured using a Hanna 209 pH meter coupled with pH and redox probes. Conductivity was measured simultaneously using a conductivity meter.

3.2.4. Enzyme assays

Dehydrogenase enzyme activity was determined by the reduction of 2,3,5-triphenyltetrazolium chloride (TPC) to triphenyl formazan (TPF), using an approach modified from Ohlinger (1995). Colour was measured at 546 nm against TPF standards, with activity expressed in $\mu\text{g TPF g}^{-1} \text{ h}^{-1}$. Cellulase activity was determined by the reduction of potassium hexacyanoferrate (III) to potassium hexacyanoferrate (II), which was reacted with ferric ammonium sulphate to form a coloured complex of ferric hexacyanoferrate (II), using carboxy-methyl cellulose as a substrate. Colour intensity was read at 690 nm, with activity expressed as mg GE (glucose equivalent) $\text{g}^{-1} \text{ day}^{-1}$ (Schinner and Vonmersi, 1990). Xylanase activity was also measured by the reduction of potassium hexacyanoferrate (III), with colour intensity assessed at 690 nm, using xylan as a substrate. Xylanase activity was expressed as mg GE (glucose equivalent) $\text{g}^{-1} \text{ day}^{-1}$ (Schinner and Vonmersi, 1990).

3.2.5. Statistical analysis

A repeated measurements ANOVA was conducted in GenStat (v15.1.) to assess differences in CO_2 and CH_4 production with treatment. Rates of CO_2 and CH_4 were log-transformed to comply with the assumption of normality for ANOVA. Species, treatment and day were included as fixed effects, with the replicate included as a blocking factor. Differences in peat physiochemical properties and enzyme activities between treatments and species were assessed by a two-way ANOVA. Conductivity, redox potential and dehydrogenase,

cellulase and xylanase activities were log-transformed. The relationship between peat properties and cumulative CO₂ and CH₄ production was assessed by applying backward stepwise elimination using multiple linear regression models. Significance was assessed at $p < 0.05$. Full ANOVA tables are presented in Appendix B.

3.3. Results

3.3.1. CO₂ production

REC addition was associated with a significant increase in CO₂ production ($F_{7,28} = 54.79$, $p < 0.001$, Figure 3.1a-d) but there was no difference in CO₂ production between species ($F_{1,4} = 0.63$, $p = 0.47$). Furthermore, there was no significant interaction between treatment and species ($F_{7,28} = 0.81$, $p = 0.59$), indicating that the extent of response to different REC solutions was not dependent on the botanical origin of the peat and associated organic matter properties. There was also no significant interaction between time following incubation, treatment and species ($F_{28,128} = 0.87$, $p = 0.61$).

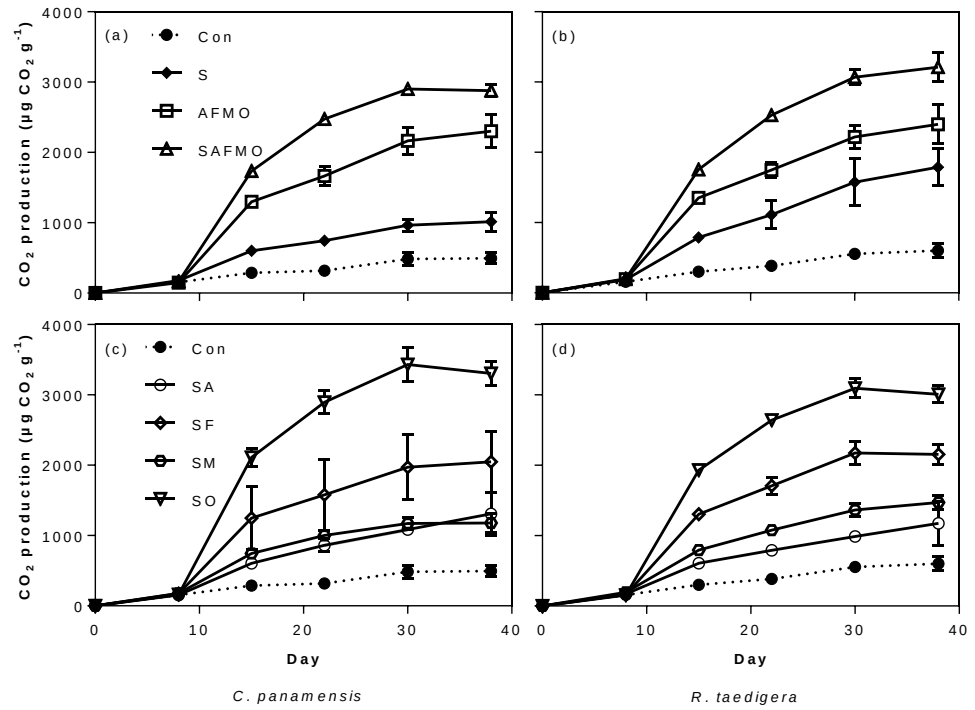


Figure 3.1: Cumulative CO₂ production from *C. panamensis* (a & c) and *R. taedigera* (b & d) in response to control (Con), sugar (S), combined organic acid (AFMO), combined sugar and organic acid additions (SAFMO) (a & b), and sugar and acetate (SA), sugar and formate (SF), sugar and malate (SM), and sugar and oxalate (SO) additions (c & d). Means $\pm$ 1 SE. (n=3).

3.3.2. CH₄ Production

REC addition significantly increased CH₄ fluxes ($F_{7,28} = 5.50$, $p < 0.001$) but there was no significant difference between species ($F_{1,4} = 3.41$, $p = 0.14$), and no significant interaction between treatment and species ($F_{7,28} = 0.15$, $p = 0.99$, Figure 3.2a-d). Immediately following the end of treatment, CH₄ production from sugars was greater than other treatments but by day 38 cumulative CH₄ production was greater in the combined sugar and organic acid treatments. In contrast to changes in CO₂ production, not all treatments increased CH₄ production compared to the control. For *R. taedigera*, both sugar and formate, and sugar and oxalate additions resulted in a decrease of 47.6% and 19.6% respectively in CH₄ fluxes compared to the control. For *C. panamensis*, sugar and formate addition also reduced CH₄ fluxes by 26.6% compared to the control.

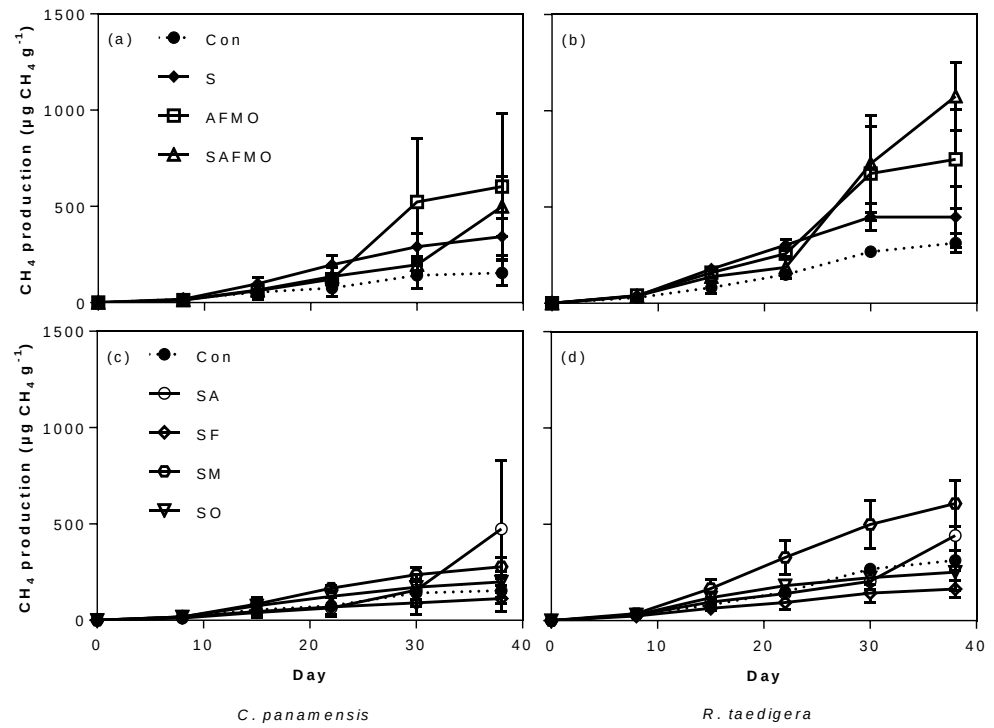


Figure 3.2: Cumulative CH₄ efflux from *C. panamensis* (a & c) and *R. taedigera* (b & d) in response to control (Con), sugar (S), combined organic acid (AFMO), combined sugar and organic acid additions (SAFMO) (a & b), and sugar and acetate (SA), sugar and formate (SF), sugar and malate (SM), and sugar and oxalate (SO) additions (c & d). Means ± 1 SE (n = 3).

3.3.3. Peat properties and enzyme assays

The majority of REC treatments were associated with significant changes in peat properties, but there were limited differences between peat types in terms *in situ* peat properties (Table 3.2).

Table 3.2: Tree characteristics and sub-surface peat properties *in situ*. Means $\pm$ 1 SE (n = 3).

Species	<i>C. panamensis</i>	<i>R. taedigera</i>
Tree height (m)	16.2 $\pm$ 0.9	10.4 $\pm$ 0.9
DBH (cm)	38.1 $\pm$ 2.7	25.0 $\pm$ 4.1
Water content (%)	86.3 $\pm$ 0.9	77.0 $\pm$ 9.5
Organic matter content (%)	90.5 $\pm$ 5.3	93.8 $\pm$ 1.4
Bulk Density (g cm ⁻³)	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0
pH	5.3 $\pm$ 0.3	5.3 $\pm$ 0.1
C:N	17.7 $\pm$ 1.2	16.8 $\pm$ 0.3

With the exception of sugar, and sugar and malate treatments, all REC additions significantly increased pH ($F_{7,30} = 53.72$, $p < 0.001$, Figure 3.3a). The sugar treatment decreased pH from 5.3 to 4.5, and combined sugar and malate addition lowered pH to 4.9. pH was generally higher under *R. taedigera*, but did not significantly differ from *C. panamensis* ($F_{1,30} = 0.07$, $p = 0.40$) and there was no significant interaction between treatment and species ($F_{7,30} = 0.25$, $p = 0.89$). REC addition significantly lowered redox potential, although sugar, and combined sugar and malate treatments showed a reduced response compared to other treatments ($F_{7,30} = 29.38$, $p < 0.001$, Figure 3.3b). Redox potential was significantly higher under *C. panamensis* (347.5 mV) than *R. taedigera* (213.8 mV) ($F_{1,30} = 4.35$, $p = 0.049$). Electrical conductivity varied significantly between treatments, but sugar and sugar and malate additions had only minimal effects compared to other REC additions ($F_{7,30} = 5.11$, $p < 0.001$,

Figure 3.3c). However, there was no difference in conductivity between species ($F_{1,30} = 2.32$, $p = 0.14$), and no significant interaction between treatment and species ($F_{7,30} = 0.57$, $p = 0.78$).

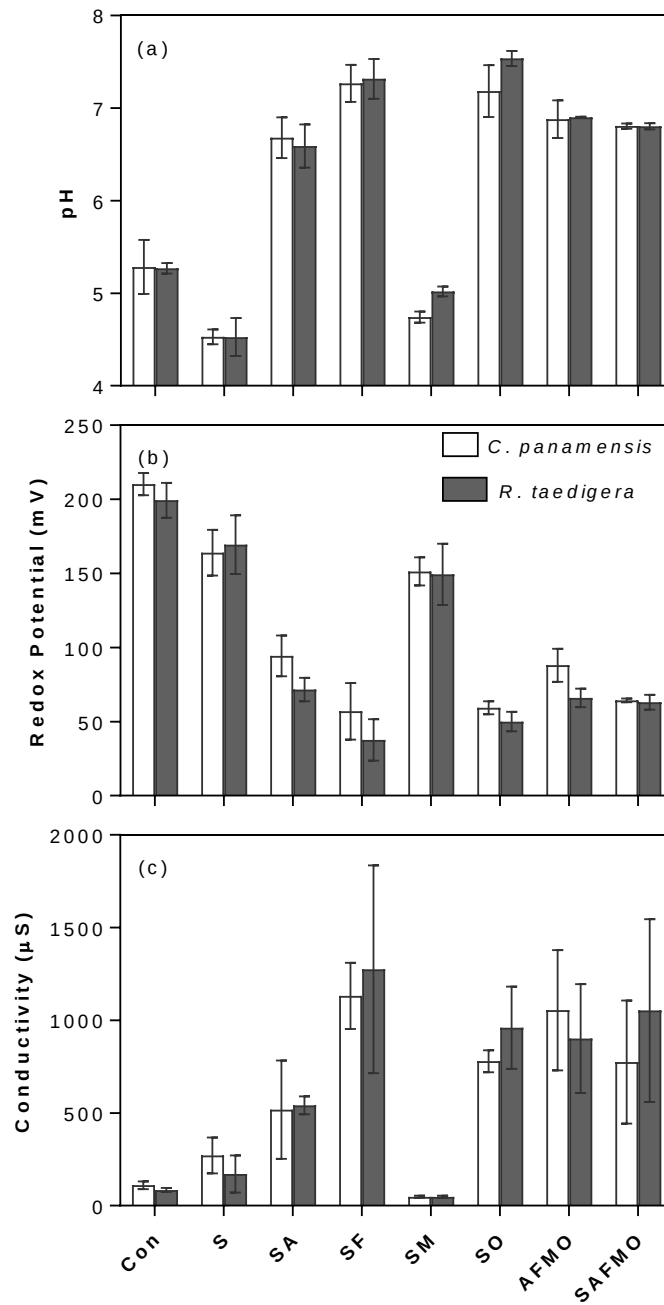


Figure 3.3: (a) pH, (b) redox potential, (c) electrical conductivity of *C. panamensis* and *R. taedigera* peats following treatment and 38 days incubation. Means ± 1 SE (n =3). Treatments are control (Con), sugar (S), sugar and acetate (SA), sugar and formate (SF), sugar and malate (SM), and sugar and oxalate (SO), combined organic acid (AFMO), and combined sugar and organic acid additions (SAFMO).

All combined treatments, with the exception of sugar and combined sugar and malate additions, significantly increased dehydrogenase activity ($F_{7,30} = 2.42$, $p < 0.001$) but there was no significant difference in activity between species ($F_{1,30} = 0.67$, $p = 0.42$), and no significant interaction effect between treatment and species ($F_{7,30} = 0.93$, $p = 0.50$). Greatest dehydrogenase activity was measured on sugar and formate addition for both *C. panamensis* (19.3 mg TPF $\text{g}^{-1} \text{h}^{-1}$) and *R. taedigera* (16.1 mg TPF $\text{g}^{-1} \text{h}^{-1}$) and lowest activity occurred for sugar treatment for *C. panamensis* (3.2 mg TPF $\text{g}^{-1} \text{h}^{-1}$) and for sugar and malate for *R. taedigera* (4.1 mg TPF $\text{g}^{-1} \text{h}^{-1}$) (Figure 3.4a). In contrast to dehydrogenase activity, there were no significant differences in cellulase or xylanase activities on REC addition (Figure 3.4b-c).

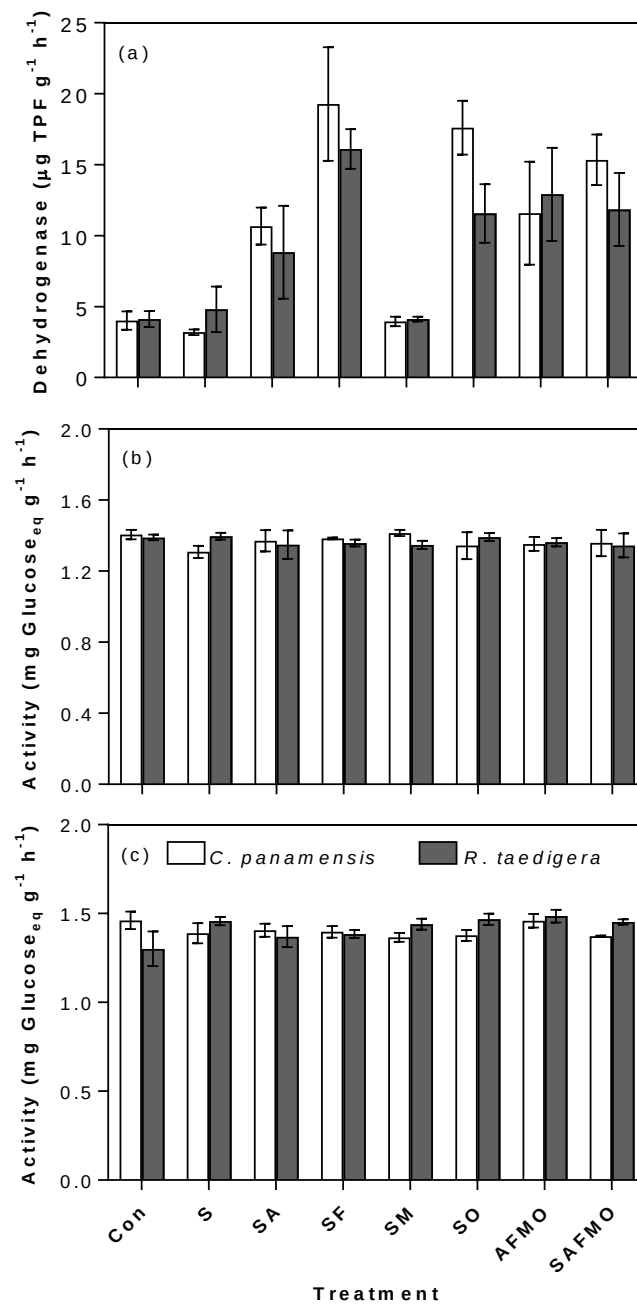


Figure 3.4: (a) Dehydrogenase, (b) Cellulase, (c) Xylanase activity of *C. panamensis* and *R. taedigera* peats following treatment and 38 days incubation. Means $\pm$ 1 SE (n = 3). Treatments are control (Con), sugar (S), sugar and acetate (SA), sugar and formate (SF), sugar and malate (SM), and sugar and oxalate (SO), combined organic acid (AFMO), and combined sugar and organic acid additions (SAFMO).

3.3.4. Linking peat properties and CO₂ and CH₄ production

No significant relationships were identified between cumulative CH₄ production and any measured peat properties. However, a significant relationship was found between cumulative CO₂ production and changes in redox potential ($T_{2,45} = 8.04$, $p = 0.01$, Figure 3.5a) and dehydrogenase activity ($T_{2,45} = 2.84$, $p = 0.05$, Figure 3.5b). In both instances, control and sugar treatments cluster together, with individual and combined organic acid additions grouped together.

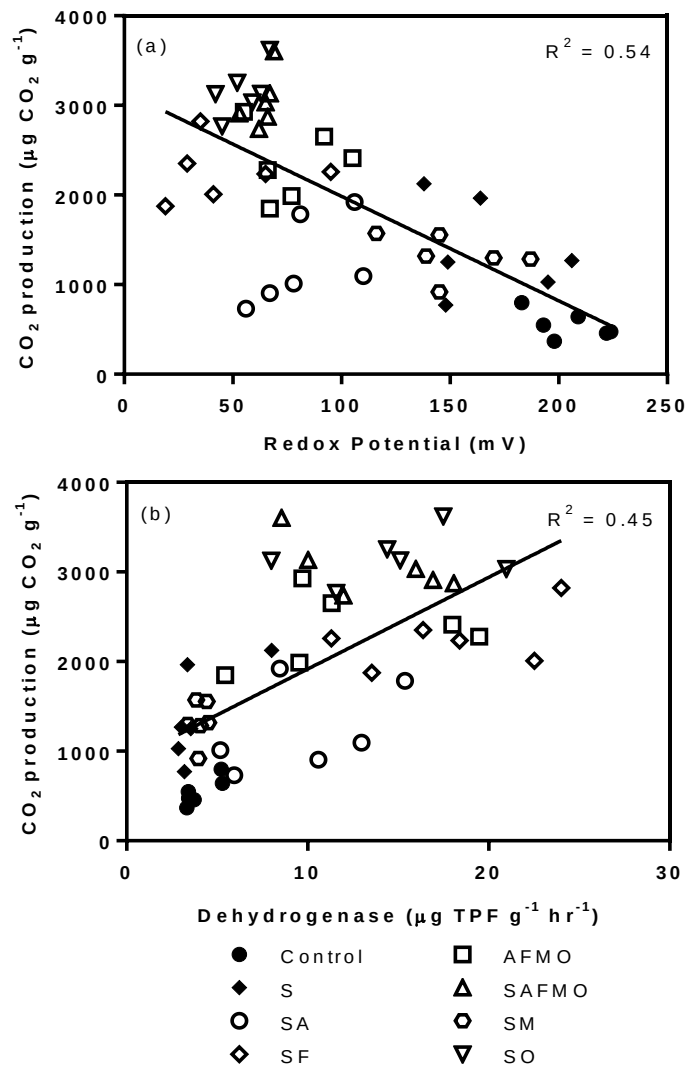


Figure 3.5: Linear regression linking changes in cumulative CO₂ evolved to changes in (a) redox potential and (b) dehydrogenase activity for all treatments. Treatments are control, sugar (S), sugar and acetate (SA), sugar and formate (SF), sugar and malate (SM), and sugar and oxalate (SO), combined organic acid (AFMO), and combined sugar and organic acid additions (SAFMO).

3.4. Discussion

3.4.1. Species influences on CO₂ and CH₄ production

The addition of labile substrates significantly increased both CO₂ and CH₄ fluxes from peat under both species. Previous *in situ* and *ex situ* work has reported differences in fluxes under *R. taedigera* and *C. panamensis*. Wright et al. (2011) found that cumulative CO₂ and CH₄ fluxes were greater from *R. taedigera* than *C. panamensis*, with strong positive relationships demonstrated between the availability of labile carbon and production. Differences in organic matter properties between subsurface peats derived from contrasting plant communities have previously been noted, including in leaf, root and shoot litter chemistries (Hoyos-Santillan et al., 2016b).

Additions of sugars, amino and organic acids have previously been found to elicit different responses from forest and agricultural soils, with changes in CO₂ production more pronounced in soils containing organic carbon of low biodegradability (Hamer and Marschner, 2005). The lack of strong differences observed between species here may therefore have arisen in this experiment because, despite previously characterised differences in peat organic matter properties across a range of depths (Hoyos-Santillan et al., 2016b), rates of decomposition (Hoyos-Santillan et al., 2015) and CO₂ and CH₄ fluxes (Wright et al., 2011), the addition of labile carbon was a more significant substrate than the soil organic matter under anoxic conditions (Hoyos-Santillan et al., 2016b). As different species are already well-established as featuring contrasting root exudate composition and concentrations (Smith, 1976), it is probable that differences in vegetation will account for small scale variation in CO₂ and CH₄

fluxes alongside differences in organic matter properties and litter properties (Hoyos-Santillan et al., 2015, Hoyos-Santillan et al., 2016b).

3.4.2. CO₂ and CH₄ response rates

Increases in CO₂ production occurred more rapidly than changes in CH₄ production during incubation, resulting in relatively high CO₂:CH₄ ratios during, and immediately, following REC solution addition. This finding mirrors similar results observed in previous anoxic incubation studies of ombrotrophic peatland samples (Avery et al., 2003, Galand et al., 2005). High ratios typically arise as, during C mineralisation, long chain polymers are hydrolysed and then fermented, producing short-chain fatty acids, alcohols, H₂ and CO₂. These products are subsequently oxidised in conjunction with the reduction of inorganic terminal electron acceptors such as nitrate (NO₃⁻) and sulphate (SO₄²⁻), and organic acceptors such as humic acids (Lovley et al., 1996, Lipson et al., 2010). These electron acceptors are used preferentially, driving CO₂ production, before CH₄ production increases through acetate oxidation and reduction (Ye et al., 2012). The preferential use of other electron acceptors may explain why after 38 days, despite its importance as a substrate for methanogenesis in peatlands, acetate addition did not increase CO₂ and CH₄ production to a greater extent than sugar addition, despite a higher total carbon input (0.3 mg C g⁻¹ day⁻¹ compared to 0.1 mg C g⁻¹ day⁻¹). The addition of acetate alone has previously been found to trigger greater peat mineralisation than the addition of the same concentration of glucose or fructose (Hamer and Marschner, 2002).

Organic anions can be readily adsorbed by soils, with increased adsorption associated with reduced mineralisation, decomposition, and microbial growth, but increased availability of phosphate (Lopez-Hernandez et al., 1986). Mineralisation rates differ depending on organic acid and soil type, the soil horizon and the presence of any additional compounds capable of forming complexes with anions, such as heavy metals (van Hees et al., 2003, Renella et al., 2006). Monovalent organic acids, which include acetate, are weakly adsorbed by soil whereas divalent organic acids, such as malate, are adsorbed to a greater extent (Jones et al., 2003). Higher rates of malate adsorption by organic matter compared to other organic acids may thus account for reduced CO₂ and CH₄ fluxes.

Sugar and formate, and sugar and oxalate additions were found to have an inhibitory effect on CH₄ production but not on CO₂ production. In the case of sugar and oxalate addition, this effect was only observed for *R. taedigera*. Organic acid additions have previously been reported to have an inhibitory effect on enzyme activities, with negative responses observed in changes to bacterial taxa diversity and abundance (Shi et al., 2011). Alternatively the responses to acetate, formate and oxalate additions may have arisen through a competition effect, with taxa that utilise organic acids as a carbon source being outcompeted by other more rapidly growing microorganisms that are better able to respond to or regulate changes in environmental conditions, such as the increase in pH (Figure 3.3a) (Paterson et al., 2007). Collectively, these results indicate that the composition of root exudates is an important regulator of greenhouse gas production, to a greater extent than the rate of input.

3.4.3. Peat properties and CO₂ and CH₄ production

Dehydrogenase assays have been widely used to indicate microbial activity in soil (Casida, 1977, Ohlinger, 1995, Shi et al., 2011). Dehydrogenase activity was generally higher than assays conducted in temperate agricultural soils (Mangalassery et al., 2015) and pine forest soils (Shi et al., 2011) but do fall in the range of the higher activities measured in other tropical soils (Chander et al., 1997, Velmourougane et al., 2013). The significant linear relationship between dehydrogenase activity and CO₂ production indicates the link between of microbial activity and CO₂ derived from microbial respiration. Higher rates of microbial activity, stimulated by labile carbon, are therefore associated with increased respiration and CO₂ production (Figure 3.5a).

Sugar addition alone did not significantly enhance dehydrogenase activity compared to the control, despite being associated with elevated CO₂ and CH₄ production. This may be due to the rapid utilisation of sugars compared to organic acid additions; enzyme activity was measured at the conclusion of the incubation, but the greatest increases in CO₂ and CH₄ production on sugar addition occurred shortly after the commencement of treatment. This rapid utilisation of sugars has previously been observed in arable ecosystems, with sugar and amino acid amendments triggering activation of soil microbial communities in less than a minute (Jones and Murphy, 2007). Previously, sugar additions were found to increase dehydrogenase activity when added at the same daily input rate, although activity was measured immediately after the cessation of two weeks treatment (Shi et al., 2011).

Previously, lower dehydrogenase activity has been associated with decreased pH (Trevors, 1984). All organic acid additions, with the exception of malate, increased pH significantly compared to the control, with sugar additions decreasing pH. Microbial degradation of carboxylic acids consumes H^+ , liberating OH^- and CO_2 (Gramss et al., 2003), while the utilisation of sugars generates H^+ (Srinivasan and Mahadevan, 2010). Increases in pH have previously been reported after organic acid additions, and such changes have been associated with significant shifts in microbial communities (Shi et al., 2011) and increases in CO_2 (Yan et al., 1996) and CH_4 production (Wang et al., 1993). Thus, changes in pH indicate likely utilisation of added substrates and imply possible differences in microbial community structure.

Redox potential significantly decreased under all organic acid treatments except under sugar and malate addition. Additions of labile plant residues can also trigger a decrease in redox potential, as high respiration causes soils to become depleted in oxygen (Figure 3.4a) (Flessa and Beese, 1995). Changes in pH, redox potential and conductivity are closely coupled because oxidation – reduction reactions frequently involve the transfer of H^+ due to changes in the oxidative state of Fe, Mn or N (Husson, 2013). Similarly, additions of labile carbon increased peat electrical conductivity for most treatments due to changes in the availability of nutrients and salts within the peat.

REC solutions did not significantly increase xylanase or cellulase activities despite the hypothesis that these extracellular enzymes would have increased activity as a result of a positive priming effect. Previously, Sjögersten, et al. (2011) found an increase in a range of extracellular enzyme activities in association with decreasing nutrient availability along a plant successional gradient that included *C. panamensis* and *R. taedigera*, with higher activities under *C. panamensis*. Activities of both enzymes were comparable to activities measured in temperate agricultural soils (Mangalassery et al., 2015).

No significant relationships were identified between soil properties and enzyme activities and cumulative CH₄ production. This suggests that while changes in CO₂ flux are partially influenced by changes in soil properties, particularly redox potential, CH₄ production is regulated by the accessibility of substrates for methanogens and the availability of terminal electron acceptors.

3.4.4. Conclusion

This study demonstrates that inputs of labile root exudate analogues are able to drive strong increases in CO₂ and CH₄ fluxes from tropical peat soils, and that the extent of this effect is more dependent on composition than concentration. A lack of difference in production of greenhouse gases between peats derived from contrasting plant species may be because the labile carbon inputs are more readily utilised by soil microbial communities than the soil organic matter. Furthermore, the input of organic anions is likely to be driving changes in measured soil properties including pH and redox potential, the latter of which was found to be an important regulator of CO₂ but not CH₄ production.

Due to the importance REC composition, we propose that *in situ* plant species composition, through species specific root exudate profiles, may contribute to small spatial variation in CO₂ and CH₄ fluxes and may partially explain the finding in Chapter 2 of higher CH₄ fluxes closer to plant stems. However, it is unclear how GHG fluxes driven by both labile carbon and organic matter decomposition are regulated *in situ* by plant inputs of oxygen, which have the potential to mitigate CH₄ production through inhibition of methanogenesis or enhancing CH₄ oxidation.

Chapter 4

Roots regulate decomposition and greenhouse gas fluxes in tropical peatlands

In previous chapters, I have demonstrated significant heterogeneity of organic matter properties within the rooting zone of two plant species commonly found in tropical peatlands, and that inputs of labile carbon, in the form of root exudate analogues, can strongly regulate production of both CH₄ and CO₂. In this chapter I assess how the presence or absence of roots and root inputs of carbon and oxygen can regulate the balance of these emissions *in situ*.

4.1. Introduction

Tropical peatlands are an important part of the global carbon cycle, containing 104.7 Gt C, and constituting a significant source of methane (CH₄), carbon dioxide (CO₂) and nitrous oxide (N₂O) emissions (Page et al., 2011, Dargie et al., 2017). CH₄ fluxes alone are estimated at 0.1–0.23 Gt CH₄ yr⁻¹, equivalent to 17 – 40% of global emissions (Laanbroek, 2010, IPCC, 2013, Sjögersten et al., 2014). Tropical peatlands are under significant threat from agricultural expansion and climate change, underlying the importance of understanding processes regulating decomposition and greenhouse gas (GHG) fluxes.

The role of environmental variables, including soil temperature (Hirano et al., 2007) and water table depth (Chimner and Cooper, 2003, Couwenberg et al., 2009, Wright et al., 2013a), in regulating GHG emissions from tropical

peatlands are relatively well understood. Vegetation type is also an important determinant of GHG emissions, as plants provide both substrates for production and a mechanism of gas transport (Colmer, 2003b, Wright et al., 2011, Pangala et al., 2013). Tropical peats are predominantly composed of decaying stem and root material, but leaf litter and root exudates also represent significant carbon inputs (Hoyos-Santillan et al., 2016b). Contrasting vegetation types are associated with differences in net emissions of GHGs, with rates of production determined by factors such as plant biomass (Liu et al., 2014) and the botanical origin of the peat (Hoyos-Santillan et al., 2016b). Moreover, differences in vegetation type are linked to differences in rooting structures (Wright et al., 2013b), which affects root respiration (Melling et al., 2013) and root inputs of exudates and oxygen (Kuz'yakov, 2006). However, the role of roots in regulating GHG emissions in tropical peatlands remains poorly understood.

Root exudates are a large and labile carbon source released by plants into the soil. Exudates include enzymes, carbohydrates, organic acids and amino acids (Badri and Vivanco, 2009) and serve a variety of functions, including microbial chemo-attraction (Walker et al., 2003) and mobilisation of nutrients (Jones and Darrah, 1994). Moreover, exudates can enhance rates of peat decomposition, most likely by enhancing carbon mineralisation (Basiliko et al., 2012) and increasing the activity of soil microbes (de Graaff et al., 2010). Rates of exudate production and root exudate profiles are known to vary among species and during the course of plant development (Gransee and Wittenmayer, 2000).

In addition to exudates, plants also release significant quantities of oxygen through roots due to radial oxygen loss. Tropical peatland vegetation is adapted to waterlogged, anoxic conditions with aerial roots, pneumatophores and aerenchymateous tissue, which enhance the transport of oxygen to roots (Cronk and Fennessy, 2001). However, despite barriers to diffusion, oxygen can still diffuse from root tissue into the soil, resulting in localised oxic conditions that can suppress CH₄ fluxes by inhibiting methanogenesis and driving methanotrophy (Watanabe et al., 1997). Moreover, oxic conditions inhibit denitrification, potentially reducing N₂O fluxes (Regina et al., 1996, Hoyos-Santillan et al., 2016a). While a variety of studies have assessed peatland methanotrophic communities (McDonald et al., 1996, Arai et al., 2014), and isotopic fractionation (Teh et al., 2006), relatively few studies report proportions of CH₄ oxidation (Yavitt et al., 1988, Pearce and Clymo, 2001).

In-growth mesocosms have been used in a variety of environments to assess fine root production, distribution and dynamics (Yuan and Chen, 2012), sources of soil CO₂ (Matvienko et al., 2014), and the impacts of arbuscular mycorrhizas on soils (Johnson et al., 2001). Studied ecosystems include arable (Steingrobe et al., 2001), grasslands (Chen et al., 2012), boreal and temperate forests (Ostonen et al., 2005, Li et al., 2013), tropical forests (Nottingham et al., 2010) and peatlands (Laiho et al., 2014). No studies have explicitly tested for differences in the effects of dominant tropical peatland plant types on peat oxygen concentrations, carbon chemistry and net GHG fluxes.

In this study we used *in situ* in-growth mesocosms to study the role of roots and root inputs of dissolved oxygen in regulating peat decomposition and GHG emissions in a mixed forest stand in a tropical peatland in Panama. We hypothesised that: i) root exclusion would significantly reduce concentrations of dissolved oxygen; ii) root exclusion would alter organic matter chemistry due to differences in labile carbon inputs and changes in root necromass; iii) changes in organic matter chemistry and root inputs of oxygen would alter the balance of GHG fluxes; iv) plants with different root structures will have contrasting oxygen inputs and resultant GHG emissions.

4.2. Methods

4.2.1. Site description

The experiment was conducted from January to May 2016 in San San Pond Sak, a large freshwater and marine wetland in Bocas del Toro Province of Panama that includes an 80 km² ombrotrophic peatland at Changuinola. The site features a central peat dome (> 8 m deep) up to 5000 years old (Phillips et al., 1997) and a distinct vegetation gradient, from *Rhizophora mangle* mangrove swamp on the coastal margins, a mixed species mangrove swamp, a palm swamp dominated by *Raphia taedigera*, a mixed species forest swamp, a monodominant *Campnosperma panamensis* forest swamp, a stunted *C. panamensis* swamp and a *Myrica-Cyrilla* bog-plain (Phillips et al., 1997). The plant communities span a strong nutrient gradient, with fertility declining towards the central peat dome (Sjögersten et al., 2011, Cheesman et al., 2012). This study was conducted in the mixed forest stand featuring both *C. panamensis* broadleaved evergreen trees and *R. taedigera* palms. *C.*

panamensis develops buttress roots to approximately 1 m depth, with lenticels for oxygen transport to roots. In contrast, *R. taedigera* forms a dense surface root mat with pneumatophores (Wright et al., 2013b).

Between 2002 and 2016 mean annual air temperature at the nearby Smithsonian Tropical Research Station on Isla Colón was 25.7 °C, with low intra-annual variability. During sampling, mean temperature was 26.9 °C. Over the same period, mean annual rainfall was 3293 mm, with a mean of 173 mm between January and May 2016. Mean sub-surface peat temperature was 25.0 °C with limited intra- and inter-annual variation. Water table height is variable, fluctuating from just above to just below the peat surface, with a range of 20 cm (Wright et al., 2013a).

4.2.2. Mesocosm design

Two mesocosm designs and one control collar were used to assess the effects of roots, their exudates and oxygen concentrations on peat decomposition and CO₂, CH₄ and N₂O fluxes. Mesocosms were constructed from 9 cm diameter PVC piping (Amanco, Mexichem Panama, S. A.) and cut to a length of 30 cm. To allow root regrowth and the transfer of exudates and oxygen, four 8 cm diameter holes were cut into the side of one mesocosm, 10 cm from the top and 5 cm from the bottom (Figure 4.1). The second treatment consisted of a solid 30 cm PVC tube to stop both root regrowth, exudate and oxygen inputs to the peat. Both were kept open at the top and bottom to allow changes in water table depth. Mesocosms were placed in holes excavated to a depth of 25 cm under five *C. panamensis* and five *R. taedigera*, within 25 cm of the trunk of

each individual plant, within a mixed forest stand, and with 5 cm remaining above the surface. Excavated peat was extracted in a circular core and placed inside the mesocosm with minimum disturbance. A depth of 25 cm was chosen as previously the majority of CO₂ and CH₄ production has been identified as occurring with 30 cm of the peat surface for both species (Wright et al., 2011). A 5 cm collar was also placed in the peat alongside the mesocosms as an undisturbed control. An additional perforated PVC tube (1.8 cm internal diameter) was inserted down the side of each mesocosm to allow measurements of water table and dissolved oxygen profiles.

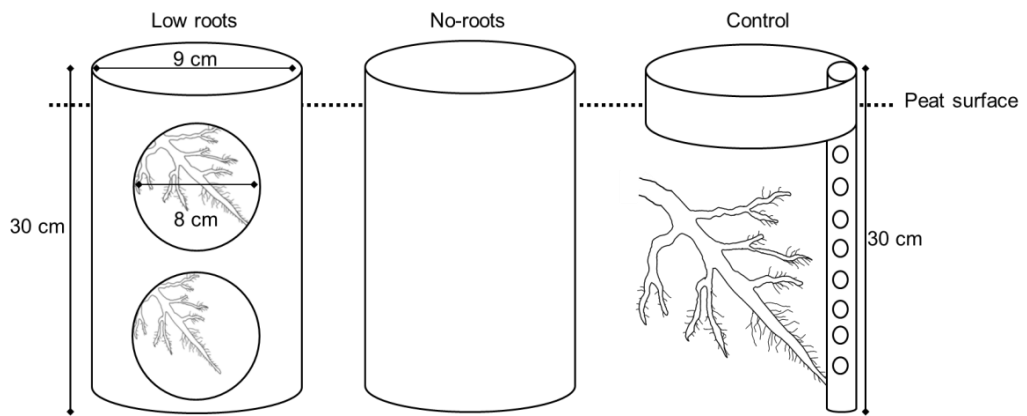


Figure 4.1: In-growth *in situ* mesocosm designs comprising root re-growth (low root), full root exclusion (no root) and a control collar.

4.2.3. Greenhouse gas fluxes

In situ GHG fluxes were measured from each mesocosm using the closed-chamber technique (Wright et al., 2011), with sampling conducted between 10 am and 4 pm each day between April – May 2016, three months following mesocosm installation. Chambers (0.35 dm³) were fitted with Suba seal[®] rubber septa (Fisherbrand, Loughborough, UK) to allow sampling. Fresh

litterfall covering the mesocosm surface was removed before placing the chamber. Gases within the headspace were mixed using a 20 ml syringe and needle and injected into pre-evacuated 12 ml glass exetainers sealed with a screw cap septum, resulting in slight over-pressure (LABCO, UK). Samples were collected over 20 minutes after fitting the chambers. Samples were shipped to the University of Nottingham for analysis. CO₂, CH₄ and N₂O concentrations were measured using a single injection system with a 1 ml sample loop using N₂ as a carrier gas through a non-polar methyl silicone capillary column (CBP1-W12-100, 0.53 mm I.D., 12 m, 5 mm; Shimadzu UK LTD, Milton Keynes, UK) and a porous polymer packed column (HayeSep Q 80/100). Thermal conductivity, flame ionization, and an electron capture detector were used to measure CO₂, CH₄ and N₂O, respectively (Hoyos-Santillan et al., 2016b). Calculations of gas flux assumed the linear accumulation of gases over time within the chamber and were calculated using the ideal gas law (Hogg et al., 1992).

4.2.4. Plant and peat properties

Plant height and diameter at breast height (DBH) were measured for each tree associated with a mesocosm. Dissolved oxygen was measured *in situ* using a combined dissolved oxygen and temperature probe (Hanna Instruments). The probe was lowered slowly and allowed to stabilise for 30 seconds at each depth prior to measurement. Measurements were made in 5 cm increments to a depth of 25 cm over three months, matching greenhouse gas sampling. Measurements were only made from the surface of the water table, as peats

were not continually inundated during sampling. Measurements were made sequentially with depth to reduce mixing of pore water.

Three months after installation, mesocosms were removed from the ground and in-growing roots were removed. A 25 cm by 8 cm core was excavated under the control collar for comparison. Living fine roots (< 2 mm diameter) were identified by their colour and condition, washed in deionised water and oven-dried at 65 °C for 3 days before weighing.

Peat sub-samples from each mesocosm were used to characterize physiochemical properties. Gravimetric moisture content was determined as mass loss following oven drying at 105 °C for 24 hours. Organic matter content was determined as mass loss after ignition for 7 hours at 550 °C. Total peat carbon (C) and total nitrogen (N) were determined from 0.2 g of dry, homogenised peat combusted using a total element analyser (Thermo Flash EA 1112, CE Instruments, Wigan, UK). As the mesocosms comprised repacked peat, bulk density was not measured for each treatment but for each plot. This was done by collecting 10 cm × 10 cm × 25 cm sections from the peat surface, and oven drying at 105 °C for 24 hours. Peat pH and redox potential were measured in a 1:5 ratio suspension of fresh peat to deionized water using a Hanna 209 pH meter coupled with pH and redox probes. Conductivity was measured simultaneously using a conductivity meter. Peat nitrate was determined using a nitrate ion electrode probe (Model CI-6735, PASCO Scientific, USA).

Ammonium (NH_4^+) was extracted from 6 g fresh peat with 40 ml 1 M KCl. Samples were placed for 1 hour on a rotary shaker, centrifuged at 2500 rpm for 5 minutes and then filtered through Whatman 42 filter paper. The filtrate (8 ml) was combined with 1 ml of phenol-nitroprusside, 1 ml of hypochlorite and 2.5 ml of de-ionised water to form a blue indophenol complex in solution. Each solution was placed in an incubator at 25 °C for 1 hour. The concentration of NH_4^+ -N was determined colorimetrically at 635 nm using a spectrophotometer.

Pore water samples were collected using 10 cm long Rhizon samplers made from hydrophilic porous polymer with a pore diameter of 0.1 μm to exclude soil particles (Rhizosphere Research Products, Wageningen, the Netherlands). Samples were frozen for storage and transport. Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were measured using a TOC-V/TN analyser (Shimadzu Corp, Kyoto, Japan).

4.2.5. Microbial biomass nutrients

Microbial biomass nutrients were determined by chloroform fumigation (Sjögersten et al., 2011). Microbial carbon and nitrogen were measured in 0.5 M K_2SO_4 extracts from peat that had been fumigated with chloroform for 24 hours. Fumigation released nutrients were calculated as the difference between fumigated and non-fumigated samples. A correction factor of 2.64 was used to account for unrecovered biomass carbon (Vance et al., 1987), and 1.85 for unrecovered nitrogen (Brookes et al., 1985).

4.2.6. Hydrolytic enzyme assays

The activity of six hydrolytic enzymes involved in phosphorus and carbon release from organic compounds were measured using fluorimetric assays using methyumbelliferone (MU) linked substrates (Marx et al., 2001). Enzymes and substrates used were: i) phosphomonoesterase: 4-methylumbelliferyl phosphate (degrades monoester-linked organic phosphates); ii) phosphodiesterase: bis-(4-methylumbelliferyl) phosphate (breakdown of nucleic acids); iii) β -glucosidase: 4-methylumbelliferyl β -D-glucopyranoside (degrades β -bonds in simple sugars); iv) N-acetyl- β -glucosaminidase: 4-methylumbelliferyl N-acetyl- β -D-glucosaminide (degrades N-glycosidic bonds); v) xylanase: 4-methylumbelliferyl β -D-xylopyranoside (degrades hemicellulose); vi) cellobiohydrolase: 4-methylumbelliferyl β -D-cellobiopyranoside (degrades cellulose).

Peat samples (2 g) were added to 200 ml of 1 mM NaN_3 to form a slurry, and stirred for 15 min. Then, 50 μl of peat suspension were dispensed into a 96-well microplate containing 100 μl of 200 mM substrate and 50 μl of acetate buffer (pH 5). This concentration of substrate is somewhat lower than the commonly used concentration of five times K_m (a measure of enzyme affinity for the substrate) which ensures enzyme saturation. This is due to indications that higher substrate concentrations penetrated cells and therefore measured internal enzyme activity. Additionally, this concentration has previously used for assaying enzyme activities in tropical peats collected from nearby sites (Sjögersten et al., 2011, Troxler et al., 2012, Hoyos-Santillan et al., 2018). Microplates were incubated at 30 °C for one (for phosphomonoesterase,

phosphodiesterase, β -glucosidase and *N*-acetyl- β -glucosaminidase) or two hours (xylanase and cellobiohydrolase). Following incubation, 50 μ l of 0.5 M NaOH was added to terminate the reaction and samples were analyzed immediately on a FLUOstar Optima microplate reader (BMG Labtech, Offenburg, Germany). Activity was calculated in nmol MU g⁻¹ min⁻¹ (Turner and Romero, 2010, Hoyos-Santillan et al., 2018).

4.2.7. Rock-Eval pyrolysis

Peat organic matter properties were assessed by Rock-Eval 6 pyrolysis configured in standard mode (Newell et al., 2016). Freeze-dried powered peat samples (60 mg) were heated to 300 °C for 3 minutes before an increase in temperature to 650 °C at a rate of 25 °C per minute in an inert N₂ atmosphere. Residual carbon was subsequently oxidized from 300 °C to 650 °C at a rate of 20 °C per minute. Released hydrocarbons were detected by flame ionisation, with an infrared cell detecting the release of CO and CO₂ during the thermal cracking of organic matter. Organic matter properties were assessed using the following selected parameters:

- Tpk2, corresponding to the temperature with the highest yield of bound hydrocarbons.
- Total organic carbon (TOC_{RE6}).
- Hydrogen Index (HI), a measure of released hydrocarbons relative to TOC_{RE6}.
- Oxygen Index (OI), corresponding to the oxygen released as CO and CO₂ relative to TOC_{RE6}.

4.2.8. Statistical analysis

Statistical analyses were carried out in Genstat v17.0. Differences between peat properties, enzyme activities, and GHG fluxes were assessed using a linear mixed effects model fitted using Residual Maximum Likelihood (REML) to account for variable dependence between mesocosms. Peat biogeochemical properties and enzyme activities included treatment and species as fixed effects and plot number as a random effect. All GHG fluxes and enzyme activities were log-transformed to meet assumptions of normality. Models of GHG fluxes additionally included sampling day as a fixed effect, and models of dissolved oxygen included sampling day and depth as fixed effects. Only fluxes measured 9 – 14 weeks following mesocosm installation were included for analysis due to disturbance effects resulting in highly elevated CO₂ fluxes, and high dissolved oxygen concentrations in treatment mesocosms. The percentage of oxidized CH₄ was calculated by comparing CH₄ effluxes from paired treatment mesocosms. Comparisons were made between treatment mesocosms rather than to the control to account for the large increase in fluxes derived from root necromass. Relationships between peat biogeochemistry, environmental variables and GHG fluxes were visualised using Principal Component Analysis based on correlation matrices.

4.3. Results

4.3.1. Vegetation and peat properties

Mean tree and palm heights and DBH were broadly comparable between species. *C. panamensis* trees had a mean height of 16.2 m and DBH of 38.2 cm, with 1951.1 g m⁻² of fine roots. *R. taedigera* palms were somewhat smaller with a mean height of 10.4 m and DBH of 25 cm and 1670.3 g m⁻² of fine roots (Table 4.1). Peats under both species had low bulk density (0.15–0.16 g cm⁻³) but high moisture content (85–86%). Organic matter content was significantly higher in *C. panamensis* peats (92%) than *R. taedigera* derived peats (88%) ($F_{1,8} = 9.83$, $p = 0.01$). While both peats were acidic, pH was significantly lower in *C. panamensis* peat (4.3) compared to *R. taedigera* (4.6) ($F_{1,8} = 7.43$, $p = 0.03$). Electrical conductivity was low (64–96 μS in controls), and peats were weakly reducing (redox potential < 300 mV). Total carbon was significantly higher in *C. panamensis* peats (40%) than *R. taedigera* peats (32%) ($F_{1,8} = 27.65$, $p < 0.001$), but there was no significant difference in total nitrogen (2%) ($F_{1,8} = 3.1$, $p = 0.12$). Ammonium and nitrate concentrations were similar between species. Microbial carbon was significantly higher in *C. panamensis* peats ($F_{1,8} = 21.11$, $p = 0.002$) but DOC was higher in *R. taedigera* peats ($F_{1,8} = 15.07$, $p = 0.01$). Mean microbial carbon and DOC were 7258 $\mu\text{g g}^{-1}$ and 563 $\mu\text{g g}^{-1}$ in *C. panamensis* control peats, and 4329 $\mu\text{g g}^{-1}$ and 642 $\mu\text{g g}^{-1}$ in *R. taedigera* control peats, respectively.

Mesocosm installation significantly reduced live root biomass ($F_{2,16} = 17.57$, $p < 0.001$). In the low root mesocosms, there were 798 g m⁻² and 343 g m⁻² under *C. panamensis* and *R. taedigera* respectively, suggesting differences in root

growth rates. No live roots were found in the no root mesocosm. Mesocosm installation did not significantly alter gravimetric moisture content ($F_{2,16} = 0.15$, $p = 0.86$) or pH ($F_{2,16} = 3.23$, $p = 0.07$). There were minimal changes in electrical conductivity and redox potential in treatment mesocosms under both species. Both total carbon ($F_{2,16} = 6.52$, $p = 0.01$) and total nitrogen ($F_{2,16} = 3.62$, $p = 0.05$) increased significantly following mesocosm installation. C:N ratios remained consistent in control plots, although were somewhat higher under both species in the low root mesocosms. There were no significant treatment effects on concentrations of ammonium, nitrate, microbial carbon and nitrogen, DOC and TDN.

Table 4.1: Mean plant and peat properties for *C. panamensis* and *R. taedigera* *in situ* mesocosms. Means $\pm$ 1 SE (n = 5).

Treatment	<i>C. panamensis</i>			<i>R. taedigera</i>		
	<i>Low roots</i>	<i>No roots</i>	<i>Control</i>	<i>Low roots</i>	<i>No roots</i>	<i>Control</i>
Height (m)	-	-	16.2 $\pm$ 0.9	-	-	10.4 $\pm$ 0.9
DBH (cm)	-	-	38.2 $\pm$ 2.7	-	-	25.0 $\pm$ 4.1
Fine roots (g m ⁻²)	798 $\pm$ 321	0	1951 $\pm$ 490	343 $\pm$ 171	0	1670 $\pm$ 417
Bulk density (g cm ⁻³)	-	-	0.16 $\pm$ 0.01	-	-	0.15 $\pm$ 0.03
Moisture content (%)	86 $\pm$ 1.0	86 $\pm$ 0.5	86 $\pm$ 1.3	85 $\pm$ 0.8	84 $\pm$ 1.1	85 $\pm$ 1.5
Organic matter content (%)	95 $\pm$ 0.5	93 $\pm$ 1.4	92 $\pm$ 2.0	89 $\pm$ 1.4	87 $\pm$ 1.1	88 $\pm$ 2.4
pH	4.3 $\pm$ 0.1	4.5 $\pm$ 0.1	4.3 $\pm$ 0.1	4.8 $\pm$ 0.1	4.8 $\pm$ 0.1	4.6 $\pm$ 0.1
Conductivity (μ S)	76 $\pm$ 20	40 $\pm$ 33	64 $\pm$ 26	81 $\pm$ 9	85 $\pm$ 18	96 $\pm$ 17
Redox (mV)	224 $\pm$ 12	224 $\pm$ 6	228 $\pm$ 6	240 $\pm$ 4	233 $\pm$ 5	240 $\pm$ 2
C (%)	44.4 $\pm$ 1.6	41.4 $\pm$ 0.9	39.9 $\pm$ 1.2	38.2 $\pm$ 1.1	42.0 $\pm$ 2.1	32.3 $\pm$ 1.9
N (%)	2.5 $\pm$ 0.1	2.4 $\pm$ 0.1	2.0 $\pm$ 0.1	2.1 $\pm$ 0.1	2.50 $\pm$ 0.2	1.9 $\pm$ 0.1
C:N	18.2 $\pm$ 0.9	17.4 $\pm$ 0.8	17.6 $\pm$ 1.0	18.1 $\pm$ 0.6	16.9 $\pm$ 0.5	16.8 $\pm$ 0.3
Ammonium (μ g g ⁻¹)	4.4 $\pm$ 0.1	5.6 $\pm$ 0.6	5.1 $\pm$ 0.4	4.9 $\pm$ 1.0	5.2 $\pm$ 0.6	4.7 $\pm$ 0.5
Nitrate (μ g g ⁻¹)	5.9 $\pm$ 1.6	7.5 $\pm$ 1.9	7.5 $\pm$ 1.3	5.1 $\pm$ 1.0	7.0 $\pm$ 1.1	4.6 $\pm$ 1.4
Microbial C (μ g g ⁻¹)	6878 $\pm$ 1531	8096 $\pm$ 865	7258 $\pm$ 906	3641 $\pm$ 458	3867 $\pm$ 554	4329 $\pm$ 475
DOC (μ g g ⁻¹)	404 $\pm$ 93	626 $\pm$ 65	563 $\pm$ 33	839 $\pm$ 81	804 $\pm$ 143	642 $\pm$ 62
Microbial N (μ g g ⁻¹)	657 $\pm$ 289	617 $\pm$ 45	624 $\pm$ 57	537 $\pm$ 97	810 $\pm$ 121	544 $\pm$ 61
TDN (μ g g ⁻¹)	1289 $\pm$ 352	2569 $\pm$ 285	2153 $\pm$ 325	2231 $\pm$ 422	1795 $\pm$ 272	2186 $\pm$ 363

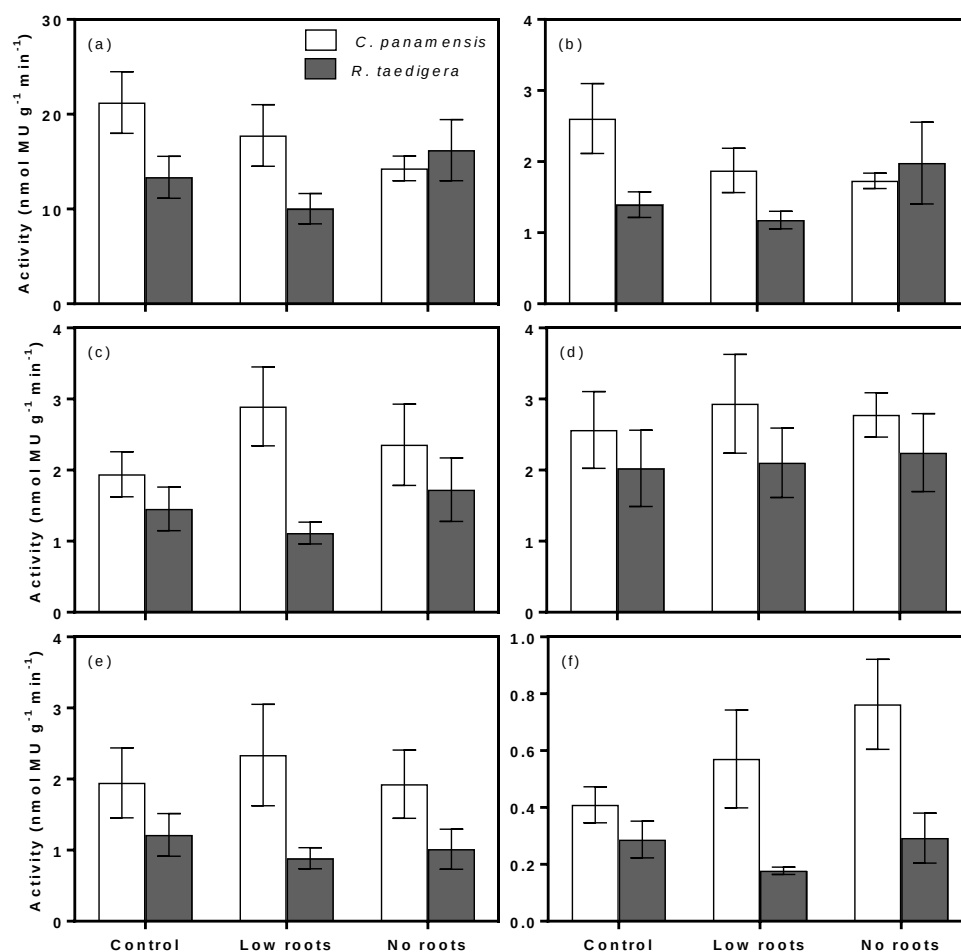


Figure 4.2: The activity of (a) phosphomonoesterase, (b) phosphodiesterase, (c) β -glucosidase, (d) *N*-acetyl- β -glucosaminidase, (e) xylanase, (f) cellobiohydrolase in experimental treatments. Means $\pm$ 1 SE (n = 5).

Most enzyme activities were greater in *C. panamensis* peat, but this difference was only significant for cellobiohydrolase activity ($F_{1,8} = 8.38$, $p = 0.02$, Figure 4.2f). Cellobiohydrolase activity was 29% greater in *C. panamensis* controls, 69% in low root mesocosms and 62% higher in no root mesocosms compared to *R. taedigera* treatments. There were no significant differences between any enzyme activities with treatment.

4.3.2. Greenhouse gas fluxes

There were no significant differences in CO₂ ($F_{1,7.7} = 5.18$, $p = 0.054$, Figure 4.3a), CH₄ ($F_{1,8} = 0.04$, $p = 0.854$, Figure 4.3b), or N₂O fluxes between species ($F_{1,7.7} = 4.9$, $p = 0.059$, Figure 4.3c), although generally mean fluxes were greater from *R. taedigera* mesocosms compared to *C. panamensis*. Treatment mesocosms were associated with a significant increase in all GHG fluxes compared to controls. Under *R. taedigera*, mean CO₂ fluxes were 37% higher in the low root mesocosm and 85% in the no root mesocosm compared to the control ($F_{2,14.8} = 7.06$, $p = 0.007$). Similarly for *C. panamensis*, fluxes were 32% higher in the low root mesocosm and 99% in the no root mesocosm. While control collar measurements indicated that the *C. panamensis* peat was a minor sink for CH₄, and *R. taedigera* peat a small source, low root and no root mesocosms for both peat types were significant sources ($F_{2,15.1} = 8.46$, $p = 0.003$). CH₄ fluxes from the low root mesocosm were 106.1 mg CH₄ m⁻² hr⁻¹ and 108.6 mg CH₄ m⁻² hr⁻¹ under *C. panamensis* and *R. taedigera* respectively, with maximum fluxes measured in the no root mesocosm under both species (642.0 mg CH₄ m⁻² hr⁻¹ and 614.9 mg CH₄ m⁻² hr⁻¹). Approximately 90 ± 4% of CH₄ was oxidized under *C. panamensis*, and 86 ± 5% under *R. taedigera*.

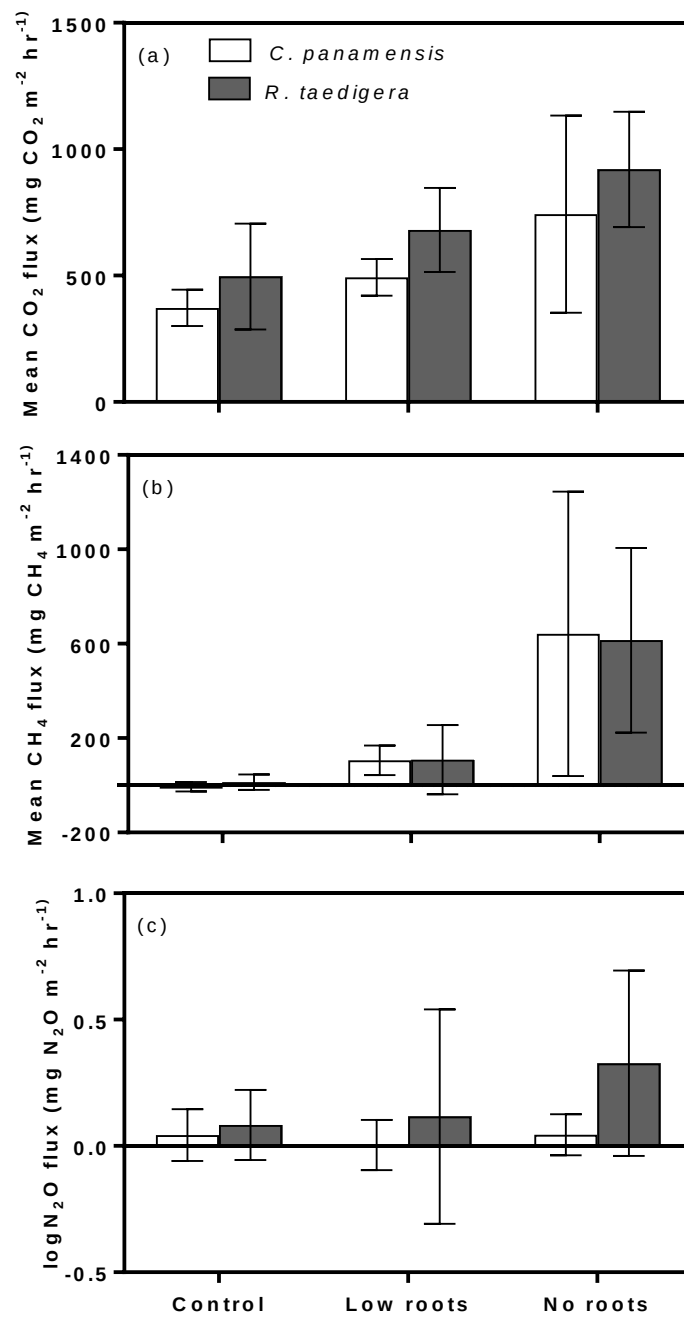


Figure 4.3: (a) CO_2 , (b) CH_4 and (c) N_2O fluxes from *C. panamensis* and *R. taedigera* in situ mesocosms and control collars. Means ± 1 SE (n = 5).

Under *R. taedigera*, N₂O emissions from no root mesocosms were 294% higher than from control collars, with a mean flux of 0.33 mg N₂O m⁻² hr⁻¹ (F_{2,15.4} = 5.59, p = 0.015). Differences in the *C. panamensis* low root mesocosm were less pronounced compared to the control, with efflux of 0.004 mg N₂O m⁻² hr⁻¹ and 0.04 mg N₂O m⁻² hr⁻¹ from the low root and no root mesocosms.

4.3.3. Dissolved oxygen profiles

Dissolved oxygen concentrations decreased significantly with under both species and for all treatments (F_{2,16} = 10.48, p = 0.001). Decreases were generally most pronounced between 5 and 10 cm depth (Figure 4.4a-b). Surface dissolved oxygen concentrations were consistently 6.5 mg l⁻¹ under *C. panamensis*, and 7.0 mg l⁻¹ under *R. taedigera*. Moreover, dissolved oxygen concentrations were significantly higher under *R. taedigera* (F_{1,8} = 29.08, p < 0.001). In *C. panamensis* control plots, concentrations declined by 8% at 25 cm relative to surface measurements, compared to a 5% decline in *R. taedigera* plots. Dissolved oxygen concentrations were significantly lower in the low root and no root mesocosm compared to the control (F_{2,16} = 10.48, p = 0.001), with the lowest dissolved oxygen concentrations measured at 25 cm depth in the *C. panamensis* no root mesocosm. Mean dissolved oxygen concentrations at 25 cm declined 1% in the *C. panamensis* low root mesocosm, and 4% in the no root mesocosm. Under *R. taedigera* mean concentrations decreased by 7% and 15% in the low root and no root mesocosms respectively, suggesting a greater net input of oxygen by *R. taedigera* roots.

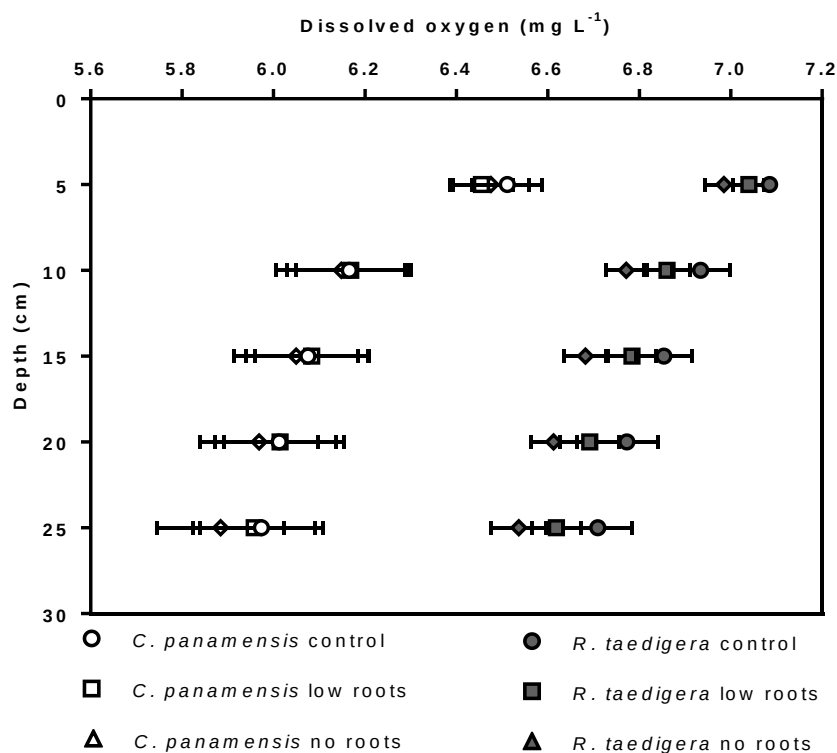


Figure 4.4: Dissolved oxygen at 5 – 25 cm depth for *C. panamensis* and *R. taedigera*. Means $\pm$ 1 SE (n = 5).

4.3.4. Organic matter characterisation

TpkS2 values were all below 420 °C for both species (Table 4.2), indicating the thermal breakdown of lignins and labile polysaccharides rather than immature humic substances (Disnar et al., 2003). Mean TpkS2 values were significantly higher under *C. panamensis* (413 °C), compared to *R. taedigera* (375 °C) ($F_{1,8} = 23.09$, $p < 0.001$). TOC_{REG} was somewhat higher in *R. taedigera* controls (37%) compared to *C. panamensis* (30%) but the overall species difference was not significant ($F_{1,8} = 0.15$, $p = 0.71$). HI was significantly higher under *C. panamensis* compared to *R. taedigera*, and effect consistent across all mesocosms ($F_{1,8} = 29.33$, $p < 0.001$, Figure 4.5) but there was no significant difference in OI between species ($F_{1,8} = 0.92$, $p = 0.36$).

TpkS2 was high and stable between treatments, ranging from 411 – 416 °C under *C. panamensis*, and 366-391 °C under *R. taedigera*. HI varied significantly between treatments ($F_{2,16} = 4$, $p = 0.04$), decreasing to 307 mg CH g⁻¹ TOC_{RE6} in the low root treatment and 320 mg CH g⁻¹ TOC_{RE6} in the no root treatment under *C. panamensis*. In contrast, in *R. taedigera* peats, the lowest HI value was found in the no root mesocosm (252 mg CH g⁻¹ TOC_{RE6}), with somewhat higher values in the low root mesocosm (263 mg CH g⁻¹ TOC_{RE6}). OI varied significantly between treatments ($F_{2,16} = 5.21$, $p = 0.02$) and in the interaction term between treatment and species ($F_{2,16} = 5.65$, $p = 0.01$). In *C. panamensis* peats, OI decreased somewhat in both treatment mesocosms, but showed a pronounced increase in *R. taedigera* mesocosms, with greatest OI found in the no root mesocosm (230 mg O₂ g⁻¹ TOC_{RE6}).

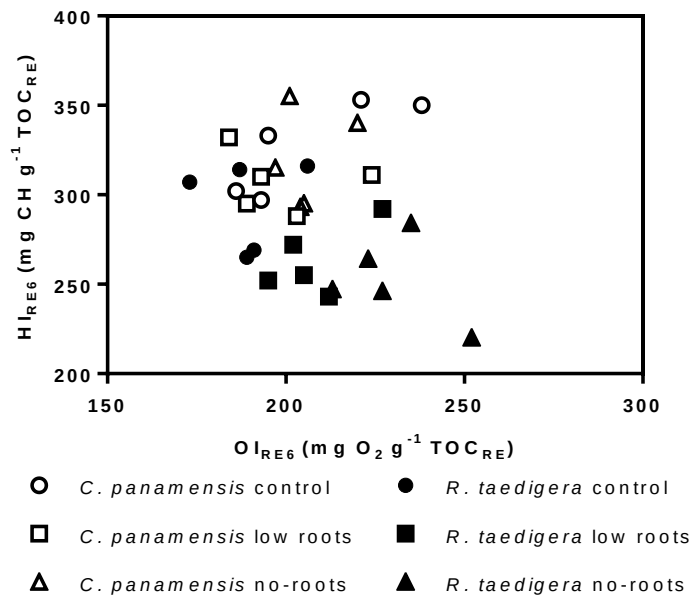


Figure 4.5: Pseudo-Van Krevelen plot relating changes in HI and OI.

Table 4.2: Rock-Eval 6 pyrolysis parameters and indices for *C. panamensis* and *R. taedigera* *in situ* mesocosms. Means $\pm$ 1 SE (n = 5).

Treatment	<i>C. panamensis</i>						<i>R. taedigera</i>					
	<i>Low roots</i>		<i>No roots</i>		<i>Control</i>		<i>Low roots</i>		<i>No roots</i>		<i>Control</i>	
TpkS2 (°C)	411	$\pm$ 10	413	$\pm$ 11	416	$\pm$ 10	366	$\pm$ 1	367	$\pm$ 0.3	391	$\pm$ 13
TOC _{RE6} (%)	41	$\pm$ 2	40	$\pm$ 0.4	30	$\pm$ 5	35	$\pm$ 2	36	$\pm$ 1	37	$\pm$ 1
HI _{RE6} (mg CH g ⁻¹ TOC _{RE6})	307	$\pm$ 8	320	$\pm$ 12	327	$\pm$ 12	263	$\pm$ 9	252	$\pm$ 11	294	$\pm$ 11
OI _{RE6} (mg O ₂ g ⁻¹ TOC _{RE6})	199	$\pm$ 7	205	$\pm$ 4	207	$\pm$ 10	208	$\pm$ 5	230	$\pm$ 7	189	$\pm$ 5

4.3.5. Peat properties and greenhouse gas fluxes

The first principal component (PC-1) separates scores by species and to a lesser extent by treatment, with distribution driven primarily by dissolved oxygen concentrations at 15 cm depth (D15), substrate availability (DOC:TDN), the extent of humification (HI:OI), and microbial C:N (Figure 4.6a-b). The second principal component (PC-2) separates scores by treatment and is driven by differences in fine roots, redox potential, and CH₄ fluxes. PCA scores show distinct clustering, both by species and treatment. Collectively PC-1 and PC-2 accounted for 52% of variance.

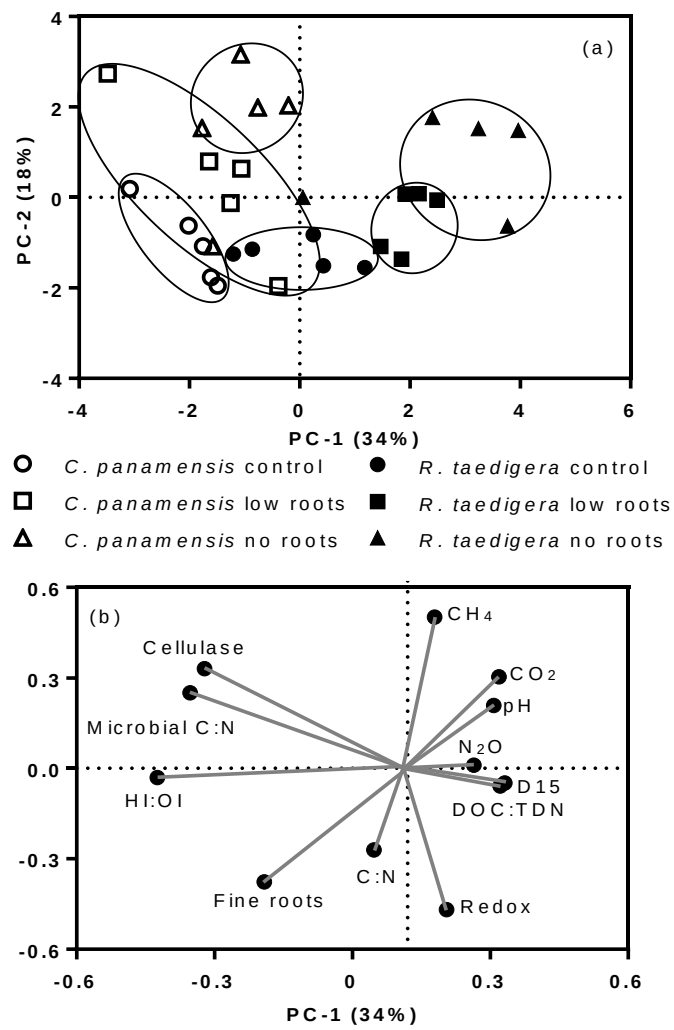


Figure 4.6: (a) Scores and (b) loadings from Principal Component Analysis for GHG fluxes and peat biochemistry. Combined PC-1 and PC-2 account for 52% of variance.

4.4. Discussion

4.4.1. Root regulation of peat biogeochemistry

Dissolved oxygen concentrations decreased markedly between 5 – 10 cm, indicating a boundary between aerobic and anaerobic decomposition processes. Significant differences in dissolved oxygen concentrations between species at depth are likely associated with differences in root structures between the species (Wright et al., 2013b). Differences in radial oxygen loss have previously been reported between wetland species, with rates determined by the anatomical features of the root cortex, epidermis and hypodermis (Pi et al., 2009). Collectively, these results indicate the importance of roots as a source of oxygen in otherwise anoxic peats, and the importance of differences in vegetation in determining oxygen inputs. The availability of oxygen in the peat profile regulates aerobic and anaerobic decomposition pathways, with decay occurring more slowly under anoxic, waterlogged conditions (Wright et al., 2013a). Oxygen from *R. taedigera* seedlings has previously been found to diffuse 2 cm from roots, suggesting that plants can have a strong role in mediating decomposition processes in the rhizosphere (Hoyos-Santillan et al., 2016a).

The marked increase in total carbon and nitrogen in the low root and no root mesocosms was most likely due to increased root necromass. There was, however, no significant increase in the activity of phosphomonoesterase and phosphodiesterase, although both are important for releasing phosphorus during decomposition. Previously, phosphomonoesterase activity was found to

be higher in the presence of fine roots and arbuscular mycorrhizas in lowland tropical forest (Nottingham et al., 2013).

In treatment mesocosms, the decline in HI, and concurrent increase in OI in *R. taedigera* mesocosms, suggests increased organic matter humification. The contrasting trends in OI between root exclusion treatments and species may be linked to previously described differences in root litter chemistry, with higher nitrogen and lignin content in *R. taedigera* litter compared to *C. panamensis* associated with enhanced decomposition under aerobic conditions (Hoyos-Santillan et al., 2015). This may partially be driven by higher root oxygen in *R. taedigera* peats, even when the peat profile was otherwise waterlogged and anoxic. Additionally, peats were not consistently waterlogged throughout the experiment due to fluctuating water tables, potentially further enhancing aerobic decomposition (Wright et al., 2013a).

4.4.2. Root regulation of greenhouse gas fluxes

The considerable increase in CH₄ flux with root exclusion highlights the importance of roots as regulators of tropical peatland GHG fluxes. The importance of oxygen diffusion from roots in suppressing CH₄ emissions has previously been shown in *R. taedigera* seedlings, with root release of oxygen suppressing fluxes by 40%. This effect was more pronounced in later growth stages of seedlings, most likely due to the increased spread of root aerenchymateous tissue enhancing the release of oxygen into peat (Hoyos-Santillan et al., 2016a). This effect mirrors that observed during rice development where CH₄ emissions decline in more advanced growth stages

due to an increase in CH₄ oxidation and the inhibition of methanogenesis (Gerard and Chanton, 1993, King, 1996). Changes in CH₄ production have been attributed to increases in microbial activity possible due to changes in temperature (Blagodatskaya et al., 2014), or the reduced oxidation of CH₄ due to decreased radial oxygen loss (Hoyos-Santillan et al., 2016a).

In the no root mesocosm, in addition to the significant increase in CH₄, there was also a large increase in CO₂. While part of this increase may be accounted for by ebullition and an increase in root necromass (Blagodatskaya et al., 2014), part may also be due to the continued oxidation of CH₄ by the surface oxic peat layer at 5 cm depth where CH₄ and oxygen profiles overlap (Watson et al., 1997). A previous study in the San San Pond Sak peatland demonstrated that, despite substantial production of CH₄ at 30 cm, surface CH₄ fluxes are substantially lowered by strong oxidation (Wright et al., 2011). Previously reported proportions of CH₄ oxidation driven by both vegetation inputs of oxygen and the surface oxic layer in temperate and boreal systems are highly variable, including 22% oxidation in a *Sphagnum* dominated peatland in northern Scotland (Pearce and Clymo, 2001), 11 – 100% in a peatland in the Appalachian Mountains (Yavitt et al., 1988), and 22.9 – 79% in the rhizosphere of some wetland plants (Schipper and Reddy, 1996, Lombardi et al., 1997).

Similarly, N₂O production is closely coupled to the availability of oxygen. N₂O production in peats by the denitrification pathway is driven by anoxic conditions, which may be enhanced by a reduction in root oxygen inputs (Khalil et al., 2004). Forest clearing has previously been found to increase N₂O production most likely due to a decrease in root oxygen loss, with dissolved oxygen concentrations accounting for 20% of the variability in *in situ* emissions (Hoyos-Santillan et al., 2016a). Previously, N₂O fluxes from peats have found to be maximised in waterlogged conditions due to the dominance of the denitrification production pathway (Pihlatie et al., 2004). N₂O fluxes from treatment mesocosms were likely also enhanced by the increased availability of organic matter derived from root necromass (Pihlatie et al., 2004).

Rates of GHG production are highly dependent on substrate availability, and both low root and no root mesocosms were associated with increased carbon and nitrogen. However, differences in HI and OI indicate changes in humification and the extent to which substrates were available for microbial use. Moreover, differences in substrate availability have previously been shown to be associated with changes in pH, which in turn is associated with shifts in microbial community structure (Shi et al., 2011, Zhang et al., 2016).

Both treatment mesocosms were associated with CH₄ and CO₂ fluxes matching previously reported ebullition events. Few studies have focussed on ebullition compared to diffusion and plant-mediated pathways, but upper estimates for ebullition include 1000 mg m⁻² hr⁻¹, equivalent to 50% of total emissions

(Christensen et al., 2003). Gas bubbles can accumulate beneath highly decomposed peat layers with release triggered by falls in atmospheric pressure driving an increase in gas volume and bubble buoyancy (Tokida et al., 2005, Comas et al., 2011), and by changes in water table (Strack and Waddington, 2007). As fluxes indicative of ebullition were limited only to treatment mesocosms and not the control collar, it is likely that mesocosms acted as nucleation sites for bubble formation, with release triggered by gas sample collection.

To account for the large CH₄ efflux derived from the decomposition of recent root necromass, calculations of CH₄ oxidation were made using fluxes measured from the low root and no root mesocosms, rather than by comparison with the control which lacked the large increase in dead root material following treatment. Part of the difference in efflux between the low root and no root mesocosms may also be driven by plant vasculature acting as an additional pathway for greenhouse gas transport (Pangala et al., 2013), although it is unclear whether aerenchymateous tissue in dead root material also provides this pathway. While this effect means that it is not possible to make a precise contribution of root derived inputs of carbon and oxygen to net fluxes, it is clear that roots have a significant capacity for driving oxidation of CH₄.

4.4.3. Conclusion

Our results demonstrate that in tropical peatlands the presence of living roots can strongly influence oxygen concentrations in peat profiles. As a consequence, root inputs of oxygen are able to exert a strong influence on CH₄ emissions through enhancing oxidation of CH₄ or inhibiting methanogenesis. We estimate that up to 90% of CH₄ release can be mitigated by root inputs of oxygen, higher than previous estimates for wetland plants (Schipper and Reddy, 1996, Lombardi et al., 1997), and amongst the first for tropical peatland ecosystems. The effects of roots on tropical peatland GHG emissions vary among plant species, which is significant in the context of global land use and climate change, as shifts in peatland plant community composition may alter regional patterns of greenhouse gas fluxes through changes in root oxygen inputs. From these findings, however, it is unclear if, in addition to oxygen inputs, plant root exudates make a measureable contribution to net CH₄ fluxes, or whether decomposition of organic matter is the dominant source of CH₄ emissions in tropical peatlands.

Chapter 5

Plant inputs of recent photosynthates drive CH₄ production and reveal microbial community function

In previous chapters, I demonstrated a critical role for oxygen inputs in regulating CH₄ fluxes *in situ*, and that the composition of root exudate analogues can regulate production *ex situ*. However, no direct relationship has been shown between plant root exudates and CH₄ fluxes. This link between recent photosynthates and CH₄ production in tropical peat therefore represents a key knowledge gap in understanding the regulation of CH₄ production.

5.1. Introduction

Tropical peatlands are a significant contributor to global methane (CH₄) emissions and an important carbon store but are subject to ongoing land use change (Page et al., 2011, Dargie et al., 2017). Plant community composition is an important regulator of surface fluxes of CH₄ and carbon dioxide (CO₂) in tropical peatlands by providing a range of substrates for microbial communities (Kao-Kniffin and Zhu, 2013), influencing peat properties (Wright et al., 2013b), and providing a mechanism of gas transportation (Pangala et al., 2013).

Under waterlogged and anoxic conditions, CH₄ is produced in wetland environments by methanogenic Archaea using a combination of organic matter (predominately derived from senesced leaf, stem and root material), and plant root exudates, which represent a significant source of carbon substrate released at depth in the peat profile (Hoyos-Santillan et al., 2016b). Concurrently, under aerobic conditions generated by roots and in inconsistently inundated surface peats, CO₂ is produced from the oxidation of CH₄ by methanotrophs, as well as through autotrophic root respiration and heterotrophic microbial respiration.

Significant diurnal variation in CO₂ fluxes from tropical peatlands in Indonesia and Panama has previously been reported, with fluxes increasing rapidly throughout daylight hours and declining overnight (Hirano et al., 2009, Wright et al., 2013b). Positive correlations have been found between diurnal changes in CO₂ efflux and air temperature, possibly due to changes in decomposition of organic matter (Hirano et al., 2009), but also with rates of photosynthesis indicating the important contribution of root exudates to the peat labile carbon pool and microbial respiration (Tang et al., 2005, Wright et al., 2013b). Similarly, there is evidence for diurnal variation in CH₄ fluxes driven by changing rates of photosynthesis in wetland plants (Thomas, 1996, Lu et al., 2004). The link between CO₂ and CH₄ fluxes and recently photosynthetically fixed carbon is likely to be species specific through a combination of differences in root exudate profiles (Smith, 1976) and oxygen inputs (Hoyos-Santillan et al., 2016a), rooting structures (Wright et al., 2013b), and contrasts in microbial community abundance and function (Joabsson et al., 1999, Kao-Kniffin and Zhu, 2013).

^{13}C pulse-labelling methodology has previously been widely used to assess the contribution of recent photosynthetically fixed carbon to net CO_2 fluxes in a range of systems including agriculture and plantation crop systems (Tavi et al., 2013, Elias et al., 2017), forests (Subke et al., 2009, Epron et al., 2011, Glaser et al., 2012), grasslands (Johnson et al., 2002, Ostle et al., 2007, De Deyn et al., 2011), temperate peatlands (Fenner et al., 2004, Ward et al., 2009, Biasi et al., 2012) and rice paddy fields (Lu et al., 2004, Pump et al., 2015). No studies, however, have been conducted that demonstrate a link between inputs derived from recent carbon fixation and CH_4 fluxes in tropical peatlands.

Previous pulse-labelling studies have demonstrated that the transfer of photosynthates to the roots and the relative contribution to net greenhouse gas (GHG) fluxes can be seasonal, and be species specific (Epron et al., 2011) as well as vary with plant development stage (Pump et al., 2015). Generally, the label is retained in above ground biomass but a sufficient proportion can be transferred to microbial pools and therefore be detected by ^{13}C phospholipid fatty acid (PLFA) analysis. Previous work has indicated that the transfer of carbon to soil microbial communities can occur within hours but is dependent on the path length (determined by plant size), and by species (Ostle et al., 2000, Epron et al., 2011, Churchland et al., 2012).

Within organic grassland soils and boreal peats, fungal biomarkers have frequently been shown to be rapidly enriched following labelling, with a decreasing ^{13}C enrichment over time, indicating the ability of fungi to rapidly

incorporate plant derived carbon (Treonis et al., 2004, De Deyn et al., 2011, Tavi et al., 2013). Significant enrichment over short timescales following labelling has also been demonstrated for Gram negative bacteria in agricultural soils (Kramer and Gleixner, 2006), and peats (Tavi et al., 2013). Rhizosphere soils have also been found to have greater abundance of Gram negative bacteria than bulk soils implying a further possible role in decomposition of labile carbon derived from recent plant inputs (Denef et al., 2009, Tavi et al., 2010). In the longer term, enrichment of Gram positive bacteria suggests an important role in the degradation of more recalcitrant organic matter (Tavi et al., 2013). Contrasts in the relative incorporation between PLFA groups between different plant communities would therefore likely indicate functional differences in the microbial communities under contrasting peat types.

This study uses a pulse-labelling $^{13}\text{CO}_2$ assay to demonstrate a link between recent photosynthetically fixed carbon and peat CH_4 fluxes. We hypothesised: i) CH_4 enrichment will differ between plant species; ii) There will be significant differences in microbial community abundance and structure between peats derived from contrasting plant species; iii) There will be significant differences in microbial community function between peats derived from contrasting plant species.

5.2. Methods

5.2.1. Field site, vegetation and peat properties

This study was conducted between February and May 2016 using plants collected from San San Pond Sak freshwater and marine wetland located in Bocas del Toro province, Panama. The wetland features an 80 km², 8 m deep ombrotrophic peatland at Changuinola initiated 4000 – 5000 years ago (Phillips et al., 1997). Coastal vegetation is dominated by *Rhizophora mangle*, followed by *Raphia taedigera* palms, mixed species forest stands, *Camposperma panamensis* broadleaved evergreen tree stands and *Myrica-Cyrilla* bog-plain. Accompanying the succession of plant communities is a strong decline in nutrient availability towards the interior of the wetland (Sjögersten et al., 2011).

Between 2002 and 2016 mean annual air temperature was 25.7 °C, with little intra-annual variability. During the period of sampling mean temperature was 26.9 °C. Over the same period, mean annual rainfall was 3293 mm, with a mean of 173 mm in February to May 2016. Mean sub-surface peat temperature is 25.0 °C. At the study sites, the water table fluctuates from just above to just below the peat surface, with a range of approximately 20 cm (Wright et al., 2013a).

Plant species were selected based on their high abundances within the forest stands at Changuinola. In addition to *C. panamensis* and *R. taedigera*, which form monodominant forest stands, *Symphonia globulifera* a second broadleaved evergreen tree was also selected for labelling. During

transplantation, there was high mortality of *C. panamensis* saplings, necessitating the selection of an additional plant species. *S. globulifera* has a tall trunk supported by buttress roots, with lenticels for root oxygenation giving it a similar physiology to *C. panamensis*.

Nine *R. taedigera*, *C. panamensis* and *S. globulifera*, selected based on similar height (30 – 40 cm) and DBH (0.3 – 0.4 cm), were collected from the mixed forest stand. Peat around the plants was removed to a 25 cm depth and in a 20 cm radius around plant stems to ensure removal of the intact root system. Plants were placed immediately in pots and transferred to the Smithsonian Tropical Research Institute research station on Isla Colón. Plants were maintained at the research station for three months prior to the beginning of labelling, including regularly watering to maintain the water table at 1 cm above the peat surface, and placing in partial shade to mimic *in situ* conditions.

5.2.2. Peat biogeochemical properties

pH, conductivity and redox potential in each pot were determined using a 1:5 ratio of peat to deionized water. Total peat carbon (C) and total nitrogen (N) were determined from 0.2 g of dry, homogenised peat combusted using a total element analyser (Flash EA 1112, CE Instruments, Wigan, UK). Moisture content was determined by through the mass of water lost from 10 g wet weight peat oven dried at 105 °C for 24 hours. Organic matter content was determined as the mass lost after ignition for 7 hours at 550 °C.

5.2.3. $^{13}\text{CO}_2$ pulse labelling assay

Labelling was conducted using custom made Perspex chambers (15.71 l) fitted with a suba seal for labelling and sampling and with a battery powered fan (Evercool EC4010M12EA) powered by 9V batteries to ensure mixing of headspace gases. Labelling was conducted between 9 am and 5 pm in direct sunlight to maximise plant photosynthesis rates. Six plants from each species were randomly selected for labelling, with three of each species retained as unlabelled natural abundance controls. The Perspex chamber was placed over the selected saplings and gently pushed into the peat to ensure a tight fit. Plants were labelled with $^{13}\text{CO}_2$ (99 atom % ^{13}C ; Cambridge Isotope Laboratories) added in pulses of 100 ml, which elevated chamber CO_2 concentration up to 6355 ppm. Chambers remained in place for 40 minutes to allow prolonged uptake of the label before lifting to allow the plants cool and condensation to dissipate. This time was chosen, as during trials, temperatures in the headspaces gradually rose over time, in some instances reaching over 40 °C. Labelling was repeated for a total of five pulses during the course of the day, beginning on 18th May 2016.

High temperatures during labelling combined with elevated CO_2 concentration may have contributed to subsequent plant mortality. Generally, increases in CO_2 concentrations from low concentrations can inhibit stomatal conductance, reducing uptake of CO_2 . However, very high concentrations (1000 – 10,000 ppm) can result in increased stomatal conductance across a range of species, and may have resulted in enhanced uptake of the label (Wheeler et al., 1999). The effects of high temperature on plant mortality were mitigated by labelling

for short periods (40 minutes) which meant that final headspace temperatures were approximately 30 °C, only somewhat elevated about ambient air temperature.

Smaller headspaces (0.37 L) were used for sampling directly from the peat surface. Duplicate headspace samples were collected one day prior to labelling, and one, three, seven and 14 days post labelling. Samples (20 ml) were collected immediately following the fitting of the headspace and after 10 and 20 minutes, and injected into 12 ml pre-evacuated glass exetainers fitted with a screw cap septum.

5.2.4. CH₄ and ¹³CH₄ isotopic analyses

CH₄ concentrations were measured by gas chromatography using a single injection system fitted with a 1 ml sample loop using H₂ as a carrier gas and a non-polar methyl silicone capillary column (GC-2014; Shimadzu, Milton Keynes, UK). CH₄ was detected using a flame ionization detector respectively. Samples that were found to be under-pressurised at the time of analysis were discarded; these duplicate samples were not subsequently analysed by GC-C-IRMS.

For determination of $\delta^{13}\text{C-CH}_4$, headspace gases were manually injected into an Isoprime Trace gas analyser coupled to an Isoprime continuous flow isotope ratio mass spectrometer (Elementar UK, Stockport) at the NERC Life Sciences Mass Spectrometer Facility, CEH Lancaster, UK. Samples were initially passed through a Magnesium perchlorate/ Carbosorb scrubber trap at 20 ml

min⁻¹ to eliminate water and CO₂. CH₄ is oxidized in a combustion furnace using a braided platinum/copper/nichrome furnace wire inside a ceramic furnace tube of 200 mm × 0.4 mm i.d. heated in a furnace at 960 °C. A preparation flow rate of 10 psi was required to give a flow rate of 20 ml min⁻¹ through the furnace at full operating temperature. The instrument was calibrated throughout the analyses with working CH₄ standards cross calibrated with a CO₂ reference gas, calibrated to NIST REF-Heavy Palaeomarine Origin (CO₂) (RM 8562) and NIST REF-Biogenic Modern Biomass Origin (CO₂) (RM 8564). $\delta^{13}\text{C-CH}_4$ was expressed in per mil (‰). The reproducibility of $\delta^{13}\text{C-CH}_4$ was better than ± 0.2 ‰.

5.2.5. PLFA and ¹³C-PLFA analysis

PLFAs were extracted from peat collected 14 days following labelling. PLFAs were extracted following the Bligh and Dyer protocol (1959). Total lipids were extracted from 500 mg of freeze-dried peats using citrate buffer (0.15 M, pH 4), 1.9 ml chloroform (CHCl₃), 3.8 ml methanol (MeOH) and 2 ml Bligh and Dyer reagent (CHCl₃: MeOH: citrate buffer; 1: 2: 0.8 volume ratio). Extracts were vortexed for one minute and left at room temperature to separate for two hours. Subsequently, extracts were centrifuged for 10 minutes at an RCF of 650 before the supernatant was transferred to a CHCl₃ rinsed glass tube. This step was repeated to ensure complete extraction of lipids from the peat pellet. Citrate buffer and chloroform (1:1 volume ratio) were added and left overnight to allow separation of aqueous and organic phases. The chloroform layer was transferred to a clean glass tube and blown dry under a stream of compressed N₂ at room temperature.

Lipids were separated using a silica solid phase extraction cartridge. The cartridge column was rinsed first with 15 ml methanol followed by 2.5 ml chloroform. The dry lipid extract was re-suspended in 0.5 ml chloroform and added to the column. Lipids were separated into neutral lipids, glycolipids and phospholipid fractions using chloroform, acetone and methanol solutions respectively. The PLFA fraction was collected and evaporated under a stream of compressed N₂ in a heating block set at 36 °C.

Phospholipid samples were re-suspended in 1 ml MeOH: toluene (1:1 volume ratio) and trans-esterified to fatty acid methyl esters (FAMES) using 1 ml 0.2 M KOH dissolved in methanol. For liquid extraction, 2 ml of hexane:chloroform (4:1 volume ratio), 0.3 ml acetic acid (1.0 M), and 2 ml ultrapure water were added. C13 and C19 internal standards were added to the samples before evaporating FAMES under a stream of compressed N₂ and re-suspension in hexane prior to GC analysis. PLFAs were identified and quantified using gas-chromatography.

Identification of individual PLFAs was carried out using gas chromatography mass spectrometry (GC-MS) using an Agilent Technologies 5973 Mass Selective Detector (electron impact ionisation 70Ev, scan mode) coupled to an Agilent Technologies 6890 GC fitted with a 50m × 0.32mm i.d. × 0.25 µm CP-Sil 5CB fused silica capillary column. The temperature program was as follows: 50 °C (5) – 150 @ 10 °C min⁻¹ – 270 @ 3 °C min⁻¹ – 320 @ 20 °C min⁻¹.

$\delta^{13}\text{C}$ values of individual PLFAs were analysed using the technique of gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS). Compounds were separated using an Agilent Technologies 6890 series gas chromatograph (splitless mode) fitted with a 50 m x 0.32 mm x 0.2 μm CP-SIL 5CB column. The temperature program was held isothermally at 50 $^{\circ}\text{C}$ for 5 min and then ramped as follows: 50 $^{\circ}\text{C}$ – 150 $^{\circ}\text{C}$ @ 10 $^{\circ}\text{C min}^{-1}$ – 270 @ 3 $^{\circ}\text{C min}^{-1}$ – 340 @ 20 $^{\circ}\text{C}$ (hold 5 min), using He_2 as the carrier gas. The GC effluent was diverted via a heart split union to a ceramic combustion furnace (650 mm $\times$ 0.3mm i.d.) packed with a copper oxide/platinum/nichrome catalyst wire heated at 940 $^{\circ}\text{C}$. Water was removed from the combustion products by a passing the effluent through a nafion membrane, prior to the CO_2 entering the Isoprime isotope ratio mass spectrometer (IRMS) (Isoprime Ltd). The PLFA $\delta^{13}\text{C}$ values were corrected for the addition of the extra carbon atom introduced to the molecule during methylation, using a correction factor obtained by CF-EA-IRMS measurement on the derivatising methanol and application of the mass balance equation (Jones et al., 1991), where N_{PLFA} is the number of carbon atoms in the PLFA molecule, $^{13}\text{C}_{\text{FAME}}$ is the $\delta^{13}\text{C}$ values of the methylated PLFA, and $^{13}\text{C}_{\text{MeOH}}$ is the $\delta^{13}\text{C}$ value of the methanol used for methylation (-37‰):

$$\delta^{13}\text{C}_{\text{PLFA}} = \frac{(N_{\text{PLFA}}+1) \times \delta^{13}\text{C}_{\text{FAME}} - \delta^{13}\text{C}_{\text{MeOH}}}{N_{\text{PLFA}}} \quad (\text{Eq. 5.1})$$

Standard PLFA nomenclature (A:B ω C) was used where A refers to the total number of carbon atoms, B refers to the number double bonds and C refers to the location of double bonds. ‘A’, ‘i’, ‘cy’, and ‘me’ refer to anteiso-, iso-,

cyclopropane and methyl groups respectively (Kong et al., 2011). C15:0i, C15:0a, C16:0i, C17:0i and C17:0a PLFA biomarkers were assigned to Gram positive bacteria. C16:1 ω 7, C17:0, C18:1 ω 7, and 7,8Cy-C19:0 were assigned to Gram negative bacteria. Fungal PLFA biomarkers were C18:2 ω 6c, C18:1 ω 9c. C14:0, C15:0, C16:1 ω 6, C16:0, C17:1 ω 8, 10Me-C16:0, C17:1, C18:0, and 10Me-C18:0 biomarkers were unclassified due to a lack of specificity to any microbial group.

5.2.6. Calculations and statistical analysis

Natural abundances of ^{13}C are typically expressed as $\delta^{13}\text{C}$ (‰), which describes the ratios (R) of ^{13}C and ^{12}C relative to the standard. $\delta^{13}\text{C}$ values were calculated as:

$$\delta^{13}\text{C} = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000 \quad (\text{Eq. 5.2})$$

Atom %, the absolute number of atoms of a given isotope in 100 atoms of an element (Ward et al., 2009), was calculated for CH_4 fluxes and PLFAs from comparison to pre-pulse measurements as:

$$\text{Atom \%} = \frac{\left(100 \times \text{AR} \times \left(\frac{\delta^{13}\text{C}}{1000} + 1\right)\right)}{\left(1 + \text{AR} \times \left(\frac{\delta^{13}\text{C}}{1000} + 1\right)\right)} \quad (\text{Eq. 5.3})$$

where AR equals the absolute ratio (0.0112372) of PDB standard material. Atom % was subsequently used to calculate ^{13}C enrichment of CH_4 fluxes

relative to unlabelled natural abundance control plants of the same species (Table 5.1). ^{13}C excess was calculated as:

$$^{13}\text{C atom (\% excess)} = \text{atom \%}_{\text{labelled}} - \text{atom \%}_{\text{natural abundance}} \quad (\text{Eq. 5.4})$$

CH_4 fluxes were calculated using the ideal gas law and assuming the linear accumulation of gases over time within the chamber (Hogg et al., 1992). CH_4 fluxes from labelled plants comprised both pre-existing natural abundance ^{13}C as well as ^{13}C derived from the pulse. Natural abundance ^{13}C was quantified using measurements from prior to labelling and from unlabelled plants, which were comparable. Excess CH_4 was calculated as CH_4 post-labelling minus mean CH_4 flux from unlabelled plants. The $^{13}\text{CH}_4$ flux (ng) was calculated using atom % data and the net flux rate.

At the conclusion of the experiment four *C. panamensis*, four *S. globulifera* plants and one *R. taedigera* palm were dead and samples collected from these pots were excluded from further analysis. However, it is unclear if the high mortality was driven by treatment as there was high plant loss during transplantation from the site. Samples from *C. panamensis* and *S. globulifera* were thus combined due to their broadly similar physiologies to assess the response of broadleaved evergreen trees for the purposes of statistical analysis. Both species feature similar rooting structures and were of similar height, DBH and biomass with no significant differences in associated peat properties. This combination of three species is subsequently referred to as plant type, on the basis of the different physiologies and morphologies between broadleaved

evergreen trees (*C. panamensis* and *S. globulifera*), and the palm (*R. taedigera*). Differences in atom % and $^{13}\text{CH}_4$ fluxes, PLFA abundances and PLFA enrichment were assessed using a linear mixed effects model. Statistical models of CH_4 fluxes included plant types and sampling day. Excess $^{13}\text{CH}_4$ fluxes were log-transformed to give normal distribution of residuals. Significance was assessed $p \leq 0.05$. All statistical analyses were conducted in GenStat (v17.1).

Table 5.1: $\delta^{13}\text{C}$ natural abundance for palm and broadleaved trees for CH_4 and PLFAs.

Component	Natural abundance $\delta^{13}\text{C}$ (‰)
CH_4	-40.22
PLFAs	-29.61

5.3. Results

5.3.1. Plant and peat properties

Plants were selected for their similar heights and DBH and as a result, aboveground and belowground biomass was fairly consistent between plant types. Aboveground and belowground biomass were 4.14 g and 2.31 g for broadleaved evergreen plants, and 6.56 g and 3.86 g for palms, respectively (Table 5.2). Peat moisture and organic matter content were consistently high in peat under both plant types with means of 87.8% and 93.9%, and 86.0% and 84.6% under broadleaved evergreens and palms, respectively. Peats were acidic with low but highly variable conductivity within plant types, and were all weakly reducing (redox < 300 mV). Peats had high total carbon (35.1 – 46.1%) and nitrogen (2.0 – 2.6%), and consistent C:N ratios between plant types (17.2 – 17.6).

Table 5.2: Plant type and peat properties of broadleaved evergreen and palm plant types. Means $\pm$ 1 SE (n = 4 and 5)

Plant type	Broadleaved evergreen	Palm
Height (cm)	36.4 $\pm$ 11.2	38.9 $\pm$ 8.2
DBH (cm)	0.33 $\pm$ 0.10	0.38 $\pm$ 0.13
Aboveground biomass (g)	4.1 $\pm$ 4.0	6.6 $\pm$ 3.6
Belowground biomass (g)	2.3 $\pm$ 1.4	3.9 $\pm$ 4.2
Peat moisture (%)	87.8 $\pm$ 2.7	86 $\pm$ 4.7
Organic matter content (%)	93.9 $\pm$ 2.2	84.5 $\pm$ 18.1
pH	4.1 $\pm$ 4.1	4.4 $\pm$ 0.3
Conductivity (μ S)	128.9 $\pm$ 52.8	96.8 $\pm$ 16.1
Redox (mV)	288.4 $\pm$ 13.8	293.5 $\pm$ 7.2
C (%)	46.1 $\pm$ 7.2	35.1 $\pm$ 5.1
N (%)	2.6 $\pm$ 0.4	2.0 $\pm$ 0.3
C:N	17.6 $\pm$ 1.6	17.2 $\pm$ 0.9

5.3.2. ^{13}C atom excess and CH_4 fluxes

CH_4 fluxes measured under both plant types showed significant enrichment 24 hours following pulse-labelling ($F_{4,26.6} = 12.9$, $p < 0.001$, Figure 5.1a), with ^{13}C atom excess decreasing over time. There was no significant difference in ^{13}C atom excess between plant types ($F_{1,7} = 0.34$, $p = 0.58$), but there was a significant interaction between plant types and number of days post-labelling ($F_{4,26.6} = 2.81$, $p = 0.045$), indicating differences in the rate of response between species.

Excess CH_4 fluxes differed significantly between different plant types ($F_{1,7} = 11.45$, $p = 0.01$). Mean fluxes from palms were significantly higher than those of broadleaved evergreen saplings (Figure 5.1b). Although fluxes were variable over time, and there was a large decline in fluxes 14 days post labelling (coinciding with a decline in ^{13}C atom excess), there was no significant difference in excess $^{13}\text{CH}_4$ fluxes over time ($F_{3,21} = 1.63$, $p = 0.21$).

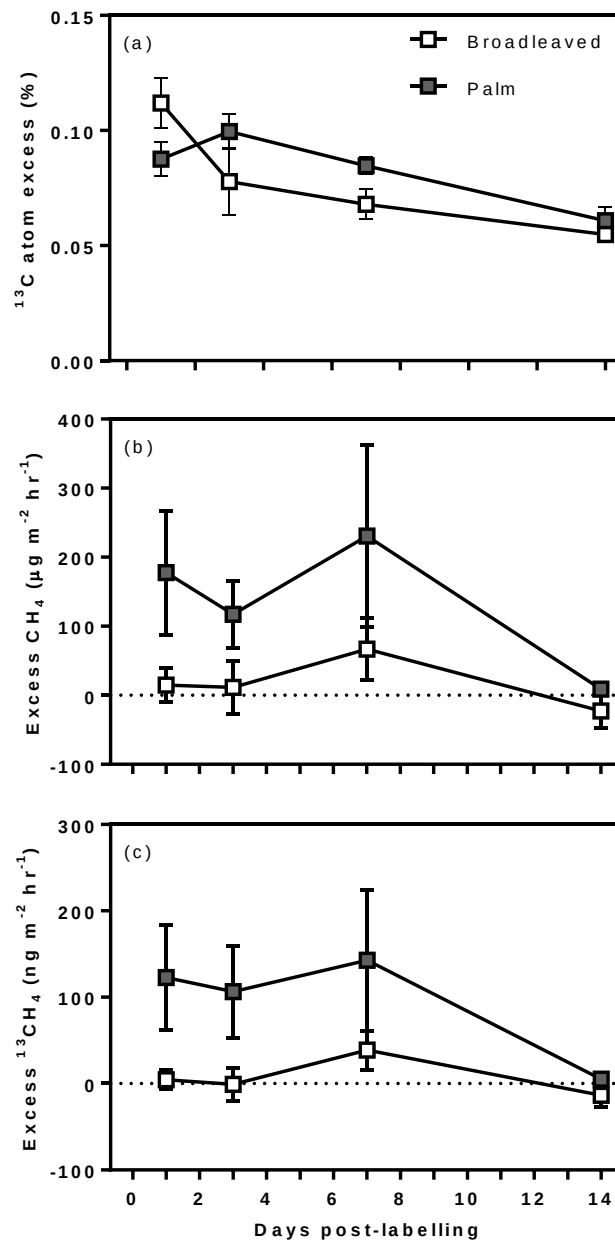


Figure 5.1: (a) Atom excess $^{13}\text{CH}_4$. (b) Excess CH_4 fluxes. (c) Excess $^{13}\text{CH}_4$ for broadleaved evergreen and palm plant types. Means ± 1 SE ($n = 4$ and 5).

Moreover, as palm fluxes were consistently higher, the interaction term between plant type and days post-labelling was also not significant ($F_{1,21} = 0.33$, $p = 0.80$). Excess $^{13}\text{CH}_4$ was significantly higher under palms ($F_{1,7} = 22.62$, $p = 0.002$, Figure 5.1c). Between one and seven days post-labelling, the

excess $^{13}\text{CH}_4$ fluxes remained consistently above $100 \text{ ng m}^{-2} \text{ hr}^{-1}$, but by day 14 declined to almost zero, through a combination of declining ^{13}C atom excess, and reduced net CH_4 fluxes.

5.3.3. PLFA and ^{13}C -PLFA

There was no significant difference in total PLFA concentrations between broadleaved and palm plant types ($F_{1,7} = 0.02$, $p = 0.90$), and no difference between fungal ($F_{1,7} = 0.11$, $p = 0.75$), total bacterial ($F_{1,7} = 0.01$, $p = 0.92$), Gram positive ($F_{1,7} = 0.0$, $p = 0.99$) or Gram negative ($F_{1,7} = 0.04$, $p = 0.85$) PLFAs (Figure 5.2b). As a percentage of total PLFAs, 8.8 – 9.4% of PLFAs were fungal, 26.9 – 28.9% were Gram positive, 39.6 – 42.5% were Gram negative, and 21.2 – 22.7% were non-specific (Figure 5.2c). Fungi-bacteria ratios were consistent between plant types (0.13), as were ratios of Gram positive to Gram negative bacteria (0.64–0.73).

There was widespread enrichment of PLFA biomarkers seven days post labelling (Figure 5.3a). There was, however, no significant difference in excess total ($F_{1,7} = 1.35$, $p = 0.28$), bacterial ($F_{1,7} = 1.02$, $p = 0.35$), fungal ($F_{1,7} = 2.87$, $p = 0.13$), Gram positive ($F_{1,7} = 1.34$, $p = 0.29$), or Gram negative ($F_{1,7} = 0.6$, $p = 0.46$) PLFAs (Figure 5.3b). There were, however, significant differences in the percentage enrichment of both Gram positive and Gram negative PLFAs relative to total PLFA enrichment (Figure 5.3c). Gram positive PLFAs were significantly more enriched for the palm ($F_{1,7} = 6.39$, $p = 0.04$), whereas Gram negative PLFAs were more enriched under the broadleaved evergreen plants ($F_{1,7} = 5.70$, $p = 0.048$).

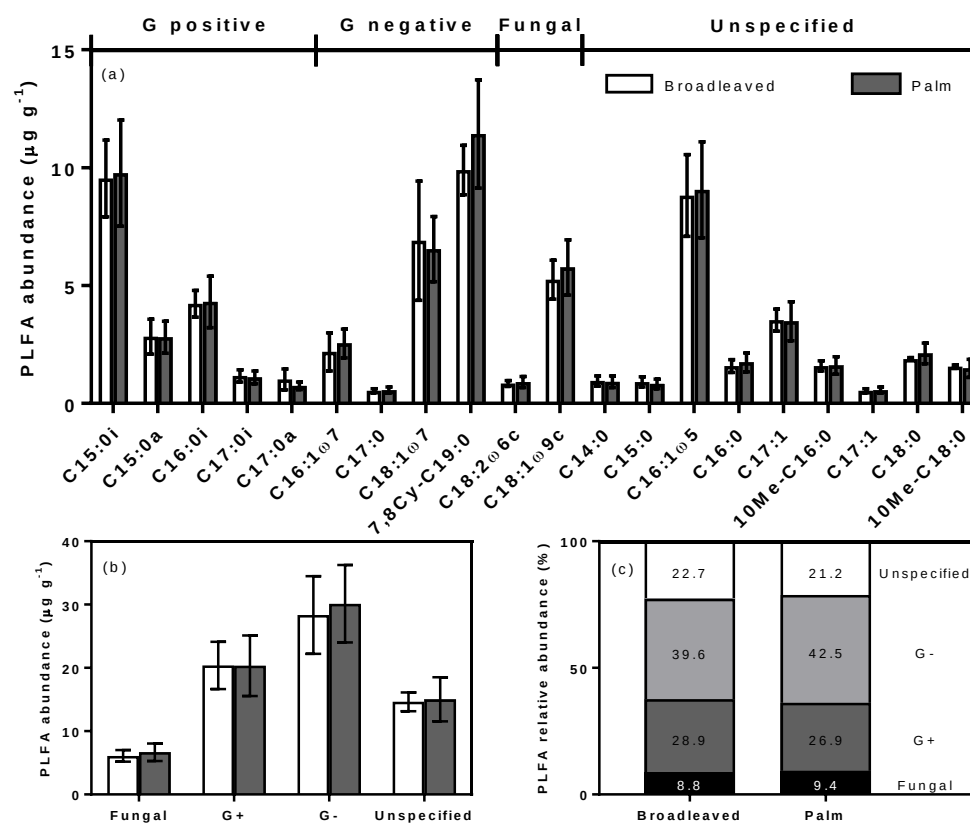


Figure 5.2: (a) Individual PLFA biomarker abundance, (b) PLFA abundance for fungal, Gram positive (G+), Gram negative (G-), and unspecified microbial groups, (c) Relative PLFA group abundance. Means ± 1 SE (n = 4 and 5).

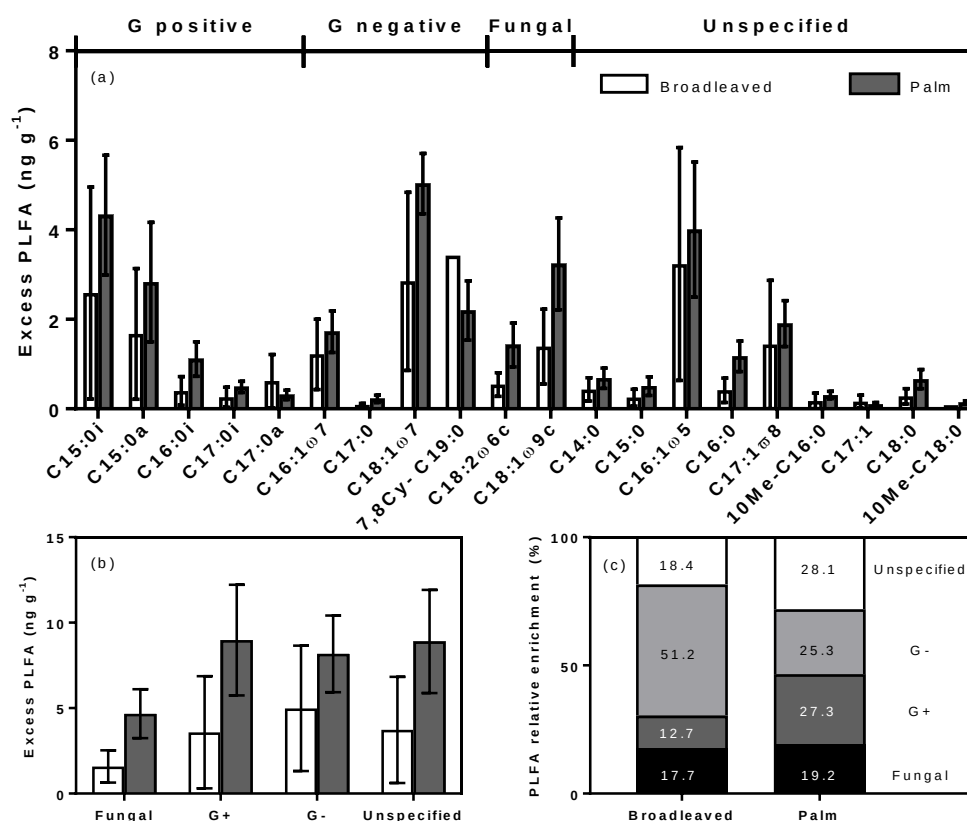


Figure 5.3: (a) Excess PLFA biomarker enrichment, (b) PLFA enrichment for fungal, Gram positive (G+), Gram negative (G-), and unspecified microbial groups, (c) Relative PLFA group enrichment. Means $\pm$ 1 SE (n = 4 and 5).

5.4. Discussion

5.4.1. Assimilation of ¹³CO₂ into CH₄

The enrichment of CH₄ fluxes with ¹³C demonstrates a conclusive link between aboveground carbon fixation and methanogenic activity in tropical peatlands. In tropical wetland ecosystems, this link has previously only been demonstrated in rice paddy soils (Cheng et al., 2008). The response was relatively rapid, with changes in $\delta^{13}\text{C}$ measured within 24 hours of labelling. This may partially be driven by the relatively short path length (all plants were less than 50 cm tall) which would allow rapid transfer of the label to the roots

prior to exudation. Mean residency time is partially dependent on plant size and height, with full grown trees having longer residency in plant tissues (Hogberg et al., 2008).

The significant interaction between plant type and number of days post-labelling suggests more rapid transfer of recently fixed carbon under the broadleaved plants compared to the palm, although the limited number of species investigated means that it is hard to ascribe any differences to a plant functional type effect rather than specific species differences (Figure 5.1a). Mean residency times in leaf tissues are known to be variable between contrasting plant functional types, such as plants vs shrubs (Carbone and Trumbore, 2007) and C4 vs C3 plants (Ueno et al., 2006). Previously, it has been suggested that different plant functional types may respond differently to changing environmental conditions within chambers during labelling and sample collection, possibly resulting in differences in assimilation of $^{13}\text{CO}_2$ (Pumpanen et al., 2004, Ward et al., 2009). Moreover, palms and broadleaved trees are known to have distinct differences in vascular tissue between monocotyledonous and dicotyledonous plants, with monocotyledonous species (which includes palms) featuring vascular bundles scattered throughout the stem, compared to a distribution around the edges of the stem in dicotyledonous species (Scarpella and Meijer, 2004). These differences may, in part, account for observed differences in fluxes, as plant vascular tissue can be a significant conduit for gas transport from the soil to the atmosphere (Pangala et al., 2013). Further differences may be due to contrasting root exudate inputs, as root exudate composition is known to vary between plant

species (Appendix A), and shown to be associated with different rates of GHG production (see Chapter 4).

5.4.2. Microbial assimilation of ^{13}C

PLFA biomarkers have been widely used to profile peat microbial community structure, as they are ubiquitous membrane spanning lipids found only in live cells and not microbial necromass (Zelles, 1999). Moreover, when combined with stable isotope labelling, ^{13}C enrichment of biomarkers is a useful tool for assessing differences in microbial community function.

Total and specific PLFA biomarker abundance was consistent between microbial communities under both plant types. Both peat types are dominated by Gram positive and Gram negative bacteria consistent with measurements made in other forest soils in Panama (Nottingham et al., 2009, Nottingham et al., 2012, Nottingham et al., 2013). Previous microbial community characterisations of peat from Changuinola have noted a dominance of *Acidobacteria* (a phylum of Gram negative bacteria) (Troxler et al., 2012), a finding also reported for other tropical (Jackson et al., 2009) and temperate peats (Dedysh et al., 2006). Fungal abundance was found to be low (8.8 – 9.4%), possibly due to the anoxic conditions arising from continual inundation by the water table (Jackson et al., 2009, Troxler et al., 2012, Chambers et al., 2016).

Differences in microbial community structure and function between contrasting vegetation types and changes in land use have previously been

reported (Bossio et al., 2005, Cookson et al., 2007). For example, Kumar et al. (2017) found significant changes in fungal to bacterial ratios on conversion of natural forest to *Jatropha curcas* plantation (Kumar and Ghoshal, 2017). Both substrate availability (C, N and P), and pH have previously been reported as key for determining community diversity (Troxler et al., 2012, Krashevskaya et al., 2015). In other instances, no overall change in community structure has been observed, despite changes in specific phyla (de Gannes et al., 2016). In this experiment, differences in specific biomarkers between peat types were also minimal (Figure 5.2a), although this does not discount the likelihood of significant differences in specialised decomposers (Hoyos Santillan et al., 2018).

Enrichment of a range of PLFA biomarkers demonstrates that the ^{13}C pulse can be utilised by a broad range of microorganisms, a finding that demonstrates that recent photosynthates are important drivers of microbial carbon dynamics in tropical peatlands as in other ecosystems (Ostle et al., 2003, De Deyn et al., 2011, Ward et al., 2012). Enrichment was most pronounced in C15:0i and C15:0a biomarkers for Gram positive bacteria, C18:1 ω 7 for Gram negative, and C18:1 ω 9c for fungi (Figure 5.3a).

The significant enrichment of Gram negative biomarkers in both peat types indicates an important role in utilising labile carbon inputs (Bird et al., 2011, Koranda et al., 2014). Gram negative biomarkers were strongly enriched relative to Gram positive biomarkers for the broadleaved plants but less so for the palm (Figure 5.3b), suggesting functional differences between microbial

communities under contrasting vegetation. PLFA biomarker enrichment was, however, measured 14 days following labelling, and therefore some enrichment of microbial groups may be driven not by direct use of root exudates but through secondary consumption of dead microbial and root biomass (Nottingham et al., 2009). Previously, Gram positive bacteria have been suggested as important in utilising more recalcitrant carbon (Kramer and Gleixner, 2006, Tavi et al., 2013), as well as assimilating label derived from dead fungal or root biomass rather than from root exudates (Denef et al., 2007, Balasooriya et al., 2008). Fungal biomarkers were also significantly enriched indicating that, despite low abundance, fungi are also important in tropical peatland decomposition processes. Previously, it has been proposed that peatland microbial communities exhibit a certain degree of functional redundancy, whereby both fungal and bacterial communities are able to utilise a similar range of substrates and drive microbial production of CO₂ (Rousk et al., 2009, Myers et al., 2012, Haynes et al., 2015). In an acidic grassland system, fungal biomarkers have previously been found to be enriched both 10 hours and 11 months following labelling, indicating their ability to utilise a diverse range of substrates in other ecosystems (Denef et al., 2009).

It should be noted that as a technique, PLFA analysis does not detect methanogenic Archaea as they contain ether-linked rather than ester-linked lipids (Gattinger et al., 2003, Palojarvi, 2005). PLFA biomarkers for methanotrophs have, however, been previously reported. PLFAs comprising C14 and C16 generally dominate type I methanotrophs (generally assumed to dominate in low CH₄ environments), whereas C18 fatty acids predominate in

type II methanotrophs (which favour higher CH₄ concentrations) (Hanson and Hanson, 1996, Singh et al., 2007). Specific type I methanotroph biomarkers include, C16:1 ω 5, C16:1 ω 7, C16:1 ω 8c and C16:1 ω 11c, and C18:1 ω 7c C18:1 ω 8c for type II methanotrophs (Roslev and Iversen, 1999, Mills et al., 2013, Maxfield et al., 2006, Singh et al., 2007). In this study both 18:1 ω 7c, and 16:1 ω 5 were identified and were present at similar abundances (Figure 5.2a), and moreover, both showed significant enrichment (Figure 5.3a) potentially indicating the presence of both type I and type II methanotrophs using enriched CH₄ (Bull et al., 2000).

5.4.3. Conclusion

In this study we have demonstrated that inputs of recent photosynthetically fixed carbon are rapidly utilised by the peat microbial community. Enrichment of CH₄ fluxes suggests that methanogenic Archaea are important users of pulses of labile carbon, the first time this has been demonstrated for tropical peatland ecosystems. Despite similar microbial community structure, there were significant differences in enrichment of Gram positive and Gram negative communities, with the former showing increased enrichment in peat under broadleaved plants. These results are of particular importance in understanding microbial community function under contrasting vegetation types, owing to differences in the extent of the response of CH₄ fluxes and microbial community enrichment. An assessment of a broader range of plant species, however, is required to fully understand the extent to which plant inputs of labile carbon vary between contrasting plant functional types.

Chapter 6

General discussion

6.1. Introduction

This study assesses the extent to which roots, and root inputs of exudates and oxygen, regulate peat biogeochemistry and greenhouse gas (GHG) fluxes in lowland tropical forested peatlands, and the extent to which these processes differ between contrasting peatland vegetation types. Globally, tropical peatlands are a significant carbon store and a major source of GHG emissions (CO_2 , CH_4 , and N_2O) but are subject to increasing pressure from land use change (Page et al., 2011, Dargie et al., 2017), and therefore understanding the role of plant inputs in regulating these processes is of particular importance.

Previous studies of CH_4 fluxes in tropical wetlands have predominantly been conducted in forested peatlands in south-east Asia and have assessed environmental regulation of fluxes (Hirano et al., 2009), seasonality (Inubushi et al., 2003b), the effects of elevated CO_2 (Inubushi et al., 2003a), and land use change (Inubushi, 1998, Melling et al., 2005, Jauhiainen, 2008). Studies that have assessed the role of roots in regulating GHG fluxes have predominantly focussed on measuring root-derived CO_2 (autotrophic respiration) with few studies on tropical ecosystems generally (Sayer and Tanner, 2010b, Nottingham et al., 2013, Matysek et al., 2017).

More recently, studies have begun to assess peatland carbon storage and distribution across central Africa (Dargie et al., 2017) and south America

(Lahteenoja et al., 2013, Kelly et al., 2017). Studies in Panama have thus far assessed variation in CO₂ and CH₄ fluxes along vegetation gradients with reference to the impacts of environmental factors (Sjögersten et al., 2011, Wright et al., 2011, Wright et al., 2013a, Wright et al., 2013b), and decomposition processes (Hoyos-Santillan et al., 2015, Hoyos-Santillan et al., 2016a, Hoyos-Santillan et al., 2016b, Hoyos Santillan et al., 2018, Utpon et al., 2018) and microbial community structure (Troxler et al., 2012). However, a significant knowledge gap remains the extent to which individual plants and their associated inputs of carbon and oxygen can regulate decomposition processes and resultant greenhouse gas fluxes.

The work presented here addresses a series of significant knowledge gaps regarding root regulation of decomposition processes in tropical peatlands. In Chapter 2, I demonstrated that peat properties within the rooting zone exhibit significant spatial heterogeneity, and these properties can vary substantially between peats derived from two contrasting plant species. In turn, changes in the size of the organic carbon pool can significantly regulate CH₄ fluxes *in situ*. In Chapter 3, I demonstrated that root exudate analogues, which can be released at significant concentrations *in situ*, can significantly regulate CO₂ and CH₄ fluxes and influence peat biogeochemical properties, thereby potentially accounting for some of the heterogeneity observed in key peat properties, such as pH and changes in labile carbon. In Chapter 4, I showed how, in addition to inputs of carbon, plant roots also release significant quantities of oxygen which can strongly regulate CH₄ production/consumption and decomposition processes. In Chapter 5, I demonstrated a conclusive link between labile

carbon derived from photosynthesis and CH₄ fluxes, and highlighted broad scale functional differences in microbial communities between broadleaved evergreen and palm plants. This chapter provides a synthesis of key findings, and context, as well as next steps for elucidating plant influences on broader patterns of nutrient cycling.

6.2. Key findings

- CH₄ but not CO₂ fluxes are significantly higher nearer to plant stems for both *C. panamensis* and *R. taedigera*, although the effect is more pronounced for *C. panamensis* (Chapter 2).
- CH₄ but not CO₂ fluxes significantly correlate to the total organic carbon pool (S2), indicating microbial use of both recalcitrant peat and also fresh leaf litter and other plant inputs (Chapter 2).
- Labile carbon additions (both sugars and organic acids) significantly increase CO₂ and CH₄ fluxes with composition, rather than quantity, determining the greatest fluxes (Chapter 3).
- Root released oxygen varies significantly between *C. panamensis* and *R. taedigera*, with increased root oxygen under *R. taedigera* (Chapter 4).
- Root oxygen can significantly mediate net CH₄ fluxes by driving the oxidation of up to 90% of net production in surface peats (Chapter 4).
- Labile carbon derived from recently photosynthetically fixed carbon significantly contributes to peat CH₄ fluxes, with different contributions from broadleaved evergreen and palm plant types (Chapter 5).

- Microbial community abundances are similar between plant types, but display significant differences in functionality demonstrated through the incorporation of the ^{13}C label in different microbial groups (Chapter 5).

6.3. Organic matter properties and greenhouse gas fluxes

Rock-Eval pyrolysis results indicated that surface peats exhibit considerable heterogeneity in organic matter properties over short distances within the rooting zone, and contrasting trends between *C. panamensis* and *R. taedigera* (Table 2.2). Additional contrasts include pH, which has previously been linked to strong differences in microbial community structure (Shi et al., 2011). Results from Chapter 3 indicate that microbial decomposition of root exudates can be significant in driving changes in both pH and redox potential (Figure 3.3), providing a possible mechanism for this finding.

Combined changes in HI and OI indicate contrasting trends in humification between *C. panamensis* and *R. taedigera* within the rooting zone (Figure 2.2), most likely driven by a combination of differences in fine root biomass, contrasts in carbon and oxygen inputs, and distinct organic matter properties. Although for *C. panamensis* CH_4 fluxes were greater at 0.5 m, differences for *R. taedigera* were much reduced possible due to larger root inputs of dissolved oxygen (Figure 4.4). Measurements of organic matter thermostability indicate that surface peats feature a large labile carbon pool comprising increased presence of lignins and polysaccharides (Disnar et al., 2003), and that this trend is fairly consistent between species (Figure 2.3). These results underline significant small scale variation in organic matter properties within the rooting

zone of individual trees of both species. It is therefore possible that peatland sites with high biodiversity will also have highly heterogeneous peat organic matter properties due to the influences of a range of different plant species (Upton et al., 2018). Overall, only measures of the total available carbon pool were linked to regulating CH₄ fluxes rather than any specific pool. Additionally, it should be noted that substantial CH₄ can also be produced at greater depth in the peat profile (Wright et al., 2011), where there is a general trend towards increasingly recalcitrant carbon alongside increases in carbon pool size (Tonks et al., 2017, Upton et al., 2018), although this production may be mitigated by oxidation in surface horizons by atmospheric diffusion of oxygen and root inputs (Figure 4.4).

6.4. The role of root adaptations

Previous studies have suggested that root adaptations, for example pneumatophores and prop roots, can enhance soil aerobic respiration (Chimner, 2004, Couwenberg et al., 2009) and concurrently reduce CH₄ emissions (Gerard and Chanton, 1993) but have never fully elucidated the mechanisms. In Chapter 4, I provide evidence that up to 90% CH₄ production can be oxidized or inhibited by root inputs of oxygen, demonstrating oxidative capacity of tropical wetland plant roots to mitigate net CH₄ efflux (Figure 4.3). Moreover, the release of root oxygen and its impact on CH₄ emissions was found to differ significantly between species even at 5 cm depth, with 7.0 mg L⁻¹ under *R. taedigera* compared to 6.5 mg L⁻¹ under *C. panamensis*. At 25 cm depth, *C. panamensis* dissolved oxygen declined by 8% compared to 5% for *R. taedigera*. These contrasts in root inputs of dissolved oxygen may be

attributable to differences in rooting structures, as *R. taedigera* features a large surface root mat (Figure 1.2c), compared to large prop roots for *C. panamensis* (Figure 1.3c). In addition, contrasts in root oxygen inputs may partially explain differences found in Chapter 2 regarding contrasts in organic matter decomposition between species at different distances from plant stems within the rooting zone. Higher oxygen inputs under *R. taedigera* may enhance aerobic decomposition processes in localised hotspots, even under otherwise anoxic, waterlogged conditions.

6.5. Root exudates regulate greenhouse gas production

The results of the $^{13}\text{CO}_2$ pulse labelling demonstrate that plant inputs of root exudates can make a measureable contribution to CH_4 fluxes, and that the extent may be species specific. These differences may be driven by a number of factors including contrasts in the rate of mass flow in the phloem (Hogberg et al., 2008), differences in photosynthetic efficiency (Carbone and Trumbore, 2007), contrasts in physiological response to labelling in chambers (Pumpanen et al., 2004, Ward et al., 2009), and distinct root exudate profiles (Smith, 1976). The results of Chapter 3 demonstrate that this last mechanism may be particularly important, as differences in composition were generally associated with significant increases in CH_4 production, and in some cases inhibition.

Moreover, the significant changes in peat properties in response to root exudate analogues, including pH and redox potential (Figure 3.3), match findings in previous studies which demonstrated that such changes can be associated with distinct microbial community profiles (Shi et al., 2011). While

PLFA analysis indicated no significant differences in microbial community abundance between plant types (Figure 5.2), there were significant differences in ^{13}C enrichment between Gram positive and Gram negative communities providing evidence of contrasts in microbial community function (Figure 5.3) (Elias et al., 2017). While the bacterial and fungal communities were enriched, PLFA analysis does not provide additional information on Archaea community structure or function. Approaches including glycerol dialkyl glycerol tetraether (GDGT) (Schouten et al., 2007, Weijers et al., 2010) and phospholipid ester lipid (PLEL) analyses (Gattinger et al., 2003) could demonstrate abundance differences between plant types but neither has been combined with stable isotope probing to assess microbial community function. Differences in root exudate inputs between species and with distance from plant stems may also account for the significant differences in pH recorded within the rooting zone (Table 2.1). Along the broader vegetation and nutrient gradient at the site, relatively small differences in pH were associated with significant differences in microbial community structure (Troxler et al., 2012) and function (Sjögersten et al., 2011).

Evidence of a species-specific link between carbon fixation, root exudation and CH_4 emissions is particularly important in the context of rising atmospheric CO_2 concentrations. A previous study measuring the effects of free-air CO_2 enrichment (FACE) on CH_4 emissions from a rice paddy over three years found increases in plant growth and CH_4 production but not oxidation (Inubushi et al., 2003a). Furthermore, as plant types in Chapter 5 displayed different responses to enrichment, it is possible that the responses of specific

plant species to CO₂ enrichment will vary, which is critically important in the context of changes in the composition of dominant peatland vegetation types, including conversion from natural forests to oil palm plantations (Tonks et al., 2017).

6.6. Implications for future research

Chapter 2, which demonstrated how variability in organic matter properties can influence GHG fluxes, has implications for future studies sampling soil organic matter and GHG fluxes under contrasting plant types, as it highlights significant spatial heterogeneity. Moreover, results from Chapter 2 and 4 also demonstrate the effectiveness of Rock-Eval pyrolysis as a technique to rapidly characterise tropical peat organic matter properties in a replicable and cost-effective manner, with results complimenting previous studies of tropical peats using tetramethylammonium pyrolysis–gas chromatography–mass spectrometry (TMAH-Py-GC/MS) (Hoyos-Santillan et al., 2015, Hoyos-Santillan et al., 2016b) and Solid-state Cross-Polarization Magic Angle Spinning Carbon-13 Nuclear Magnetic Resonance (CP/MAS ¹³C-NMR) (Wright et al., 2011). Rock-Eval pyrolysis therefore has the potential to be more widely applied in assessing organic matter properties in tropical peats, with possible applications including understanding changes in organic matter properties in peat profiles, assessing further contrasts in peats derived from different plant species, and investigating the impacts of land conversion.

Chapter 3 identified that a range of sugars and organic acids can significantly accelerate the production of CO₂ and CH₄, but responses were similar between

peats derived from contrasting botanical origins. Future studies in this area could utilise plant species specific root exudate profiles, as there remains significant uncertainty in composition for tropical plant species generally, and for palms specifically. Additionally, as amino acids are frequently identified as root exudate components, future studies should also assess their impact on N₂O fluxes.

Chapter 4 demonstrated that root oxygen is important in regulating CH₄ and N₂O fluxes by driving methanotrophy and suppressing methanogenesis, and regulating the balance of nitrification and denitrification pathways. Further assessment is needed, however, to fully separate the relative contributions of root oxygen versus oxidation at the surface oxygen layer.

Chapter 5 demonstrated a strong link between recent photosynthetically derived carbon and CH₄ fluxes. Having demonstrated this important link, future studies should assess the extent to which elevated atmospheric CO₂ concentrations have the potential to enhance CH₄ emissions through increases in input of labile exudates (Inubushi et al., 2003a). Moreover, while PLFA demonstrated functional differences in Gram positive and Gram negative bacterial communities between plant types, it did not reveal whether there are also differences in methanogenic Archaea abundance or function. Future studies could use similar ¹³C labelling methodology and test the enrichment of GDGTs, membrane spanning lipids found in both Archaea and bacteria (Schouten et al., 2007).

Appendix A

Table A1: Linear mixed effects models assessing differences in plant, soil and pore water properties. Significance was assessed at $p < 0.05$.

	Distance				Species				Distance * Species			
	F-statistic	d.f.	p	SED	F-statistic	d.f.	p	SED	F-statistic	d.f.	p	SED
Height		-			3.67	1,10	0.08	3.48		-		
DBH		-			6.27	1,10	0.03	4.57		-		
Fine root density	3.35	1,10	0.10	1.13	1.05	1,10	0.33	2.03	0.12	1,10	0.73	2.08
Bulk density	0.31	1,10	0.59	0.03	0.67	1,10	0.43	0.03	1.94	1,10	0.19	0.04
Soil moisture content	1.43	1,10	0.26	0.48	0.62	1,10	0.45	0.98	2.09	1,10	0.18	0.95
pH	5.16	1,10	0.049	0.06	1.30	1,10	0.28	0.12	1.17	1,10	0.30	0.12
Conductivity	0.27	1,10	0.62	13.6	3.21	1,10	0.10	25.9	0.02	1,10	0.89	25.9
Redox potential	0.08	1,10	0.78	3.85	0.22	1,10	0.65	7.62	0.64	1,10	0.44	7.51
Log dehydrogenase	2.86	1,10	0.12	1.07	0.18	1,10	0.68	1.07	1.93	1,10	0.20	0.10
Total carbon	0.80	1,10	0.39	0.85	1.07	1,10	0.33	4.60	0.27	1,10	0.62	3.52
Total nitrogen	0.45	1,10	0.52	0.11	0.27	1,10	0.612	0.32	0.05	1,10	0.82	0.27
C:N	0.04	1,10	0.86	0.58	1.18	1,10	0.30	0.74	1.29	1,10	0.28	0.82
Organic matter content	10.8	1,10	0.01	0.84	4.16	1,10	0.07	2.40	1.91	1,10	0.20	2.09
DOC	0.10	1,10	0.76	20.4	0.95	1,10	0.35	28.5	0.27	1,10	0.61	32.9
TDN	0.00	1,10	0.98	2.08	0.00	1,10	0.99	3.74	0.27	1,10	0.61	3.84
E₄₆₅:E₆₆₅	1.75	1,10	0.22	0.64	0.19	1,10	0.68	0.85	0.86	1,10	0.38	1.01

Table A2: Linear mixed effects models assessing differences in greenhouse gas fluxes between species and with distance from plant stems. Significance was assessed at $p < 0.05$.

	F-statistic	d.f.	p	S.E.D
logCO₂				
<i>Day</i>	7.32	4,33.8	<0.001	0.12
Distance	0.02	1,37.6	0.88	0.06
Species	0.05	1,10.2	0.83	0.09
Distance*Day	0.24	4,38.7	0.91	0.15
Species*Day	2.21	4,34.3	0.09	0.18
Distance*Species	0.9	1,39.0	0.35	0.10
Distance*Species*Day	0.8	0.8,40.3	0.53	0.22
logCH₄				
<i>Day</i>	3.92	4,29.5	0.01	0.15
Distance	6.03	1,35	0.02	0.09
Species	0.06	1,9.9	0.82	0.16
Day*Distance	0.65	4,35.9	0.63	0.21
Day*Species	1.25	4,30.5	0.31	0.23
Distance*Species	0.73	1,36.3	0.40	0.17
Day*Distance*Species	0.89	4,37.3	0.48	0.31

Table A3: Linear mixed effects models assessing differences in organic matter properties between species and with distance from plant stems. Significance was assessed at $p < 0.05$.

	Distance				Species				Distance*Species			
	F-statistic	d.f.	p	S.E.D	F-statistic	d.f.	p	S.E.D	F-statistic	d.f.	p	S.E.D
S1	0.06	1,10	0.81	2.43	0.09	1,10	0.77	2.47	1.12	1,10	0.32	3.46
S2	0.27	1,10	0.62	7.36	0.03	1,10	0.87	7.72	1.34	1,10	0.27	10.6
S3CO	0.39	1,10	0.55	1.14	4.97	1,10	0.049	1.33	4.92	1,10	0.049	1.71
S3CO₂	2.63	1,10	0.14	4.26	5.16	1,10	0.049	5.14	0.95	1,10	0.35	6.46
TpkS2	0.02	1,10	0.90	5.93	0.25	1,10	0.63	6.80	2.26	1,10	0.16	8.81
TOC_{RE}	0.66	1,10	0.44	2.43	0.62	1,10	0.45	2.15	0.10	1,10	0.76	3.31
HI	0.17	1,10	0.69	12.2	0.75	1,10	0.41	12.4	1.51	1,10	0.25	17.3
OI	0.71	1,10	0.42	8.89	6.37	1,10	0.03	8.92	2.36	1,10	0.16	12.6
logC_l	0.53	1,10	0.49	1.02	2.35	1,10	0.16	1.05	6.39	1,10	0.03	1.05
logC_i	16.52	1,10	0.002	1.07	8.42	1,10	0.02	1.10	8.60	1,10	0.02	1.12
logC_p	14.28	1,10	0.004	1.07	3.92	1,10	0.08	1.07	0.32	1,10	0.59	1.12

Appendix B

Table B1: Occurrence of common sugars in plant species root exudate profiles.

Exudates were collected through a variety of techniques and under a range of environmental conditions. a = (Grayston et al., 1997); b = (Gaworzewska and Carlile, 1982).

Species	Habitat	Glucose	Fructose	Sucrose	Rhamnose	Arabinose	Ribose	Maltose	Galactose	Xylose	Raffinose
<i>Acer saccharum</i> ^a	Temperate	Y	Y	Y	Y		Y				
<i>Betula alleghaniensis</i> ^a	Temperate	Y	Y	Y			Y				
<i>Eucalyptus callophylla</i> ^a	Sub-tropical	Y	Y	Y					Y	Y	Y
<i>Eucalyptus marginata</i> ^a	Sub-tropical	Y	Y	Y					Y	Y	Y
<i>Eucalyptus pilularis</i> ^a	Sub-tropical	Y	Y	Y	Y	Y		Y	Y	Y	
<i>Fagus grandifolia</i> ^a	Temperate	Y									
<i>Pinus banksiana</i> ^a	Boreal	Y									
<i>Pinus lambertiana</i> ^a	Boreal	Y	Y	Y	Y	Y					
<i>Pinus radiata</i> ^a	Boreal	Y									
<i>Pinus resinosa</i> ^a	Boreal	Y				Y					
<i>Pinus rigida</i> ^a	Boreal	Y		Y	Y	Y					
<i>Pinus strobus</i> ^a	Boreal	Y				Y					
<i>Pisum sativum</i> ^b	Temperate	Y	Y	Y			Y	Y		Y	Y
<i>Robinia pseudoacacia</i> ^a	Temperate	Y		Y	Y	Y					
Total:	14	14	7	9	5	6	3	2	3	4	3

Table B2: Organic acids in root exudate profiles. NS - concentration not specified. a = (Strobel, 2001); b = (Grayston and Campbell, 1996); c = (Smith, 1970); d = (Smith, 1976); e = (Sandnes et al., 2005); f = (Malajczuk and Mccomb, 1977); g = (Silva et al., 2004); h = (Strobel et al., 2001); i = (Lu et al., 2007); j = (Xie et al., 2013); k = (Krzyszowska et al., 1996); l = (Bowen, 1969); m = (Agnihotri and Vaartaja, 1967); n = (Slankis et al., 1964).

Species	Habitat	Acetate	Aconitate	Butanoate	Citrate	Coumarate	Formate	Fumarate	Gallate	Glycolate	Lactate	Maelate	Malate	Malonate	Oxalacetate	Oxalate	Phthalate	Propionate	Succinate	Tartarate	Valerate
<i>Abies grandis</i> ^a	Temperate	Y		Y	Y		Y					Y	Y		Y			Y	Y		Y
<i>Acer saccharum</i> ^{bcd}	Temperate	Y			Y									Y	Y	Y			Y		
<i>Betula alleghaniensis</i> ^b	Temperate	Y	Y		Y			Y					Y			Y			Y		
<i>Betula pendula</i> ^e	Temperate	Y		Y	Y		Y				Y		Y			Y	Y	Y	Y		
<i>Eucalyptus callophylla</i> ^{bf}	Sub-tropical				Y								Y						Y		
<i>Eucalyptus cloeziana</i> ^g	Sub-tropical	Y			Y		Y						Y			Y					
<i>Eucalyptus dunii</i> ^g	Sub-tropical	Y			Y		Y						Y			Y					
<i>Eucalyptus globulus</i> ^g	Sub-tropical	Y			Y		Y						Y			Y					
<i>Eucalyptus grandis</i> ^g	Sub-tropical	Y			Y		Y						Y			Y					
<i>Eucalyptus marginata</i> ^{bf}	Sub-tropical				Y								Y	Y					Y		
<i>Eucalyptus pilularis</i> ^b	Sub-tropical				Y								Y						Y		
<i>Eucalyptus saligna</i> ^g	Sub-tropical	Y			Y		Y						Y			Y					
<i>Eucalyptus urophylla</i> ^g	Tropical	Y			Y		Y						Y			Y					
<i>Fagus grandifolia</i> ^a	Temperate	Y						Y					Y		Y	Y			Y		Y
<i>Fagus sylvatica</i> ^{bh}	Temperate	Y	Y	Y	Y		Y					Y	Y		Y			Y	Y		
<i>Kandelia candel</i> ⁱ	Sub-tropical	Y		Y	Y		Y				Y	Y									
<i>Kandelia obovata</i> ^j	Sub-tropical	Y			Y		Y	Y			Y	Y	Y			Y			Y	Y	
<i>Picea abies</i> ^{abeh}	Boreal	Y	Y	Y	Y	Y	Y	Y	Y		Y	Y	Y		Y	Y	Y	Y	Y	Y	Y
<i>Picea rubes</i> ^{ak}	Boreal	Y			Y		Y								Y		Y				
<i>Pinus banksiana</i> ^b	Boreal	Y						Y								Y			Y		
<i>Pinus lambertiana</i> ^b	Boreal	Y												Y		Y			Y		
<i>Pinus radiata</i> ^{bk}	Boreal	Y								Y						Y			Y		
<i>Pinus resinosa</i> ^{bl}	Boreal	Y														Y					
<i>Pinus rigida</i> ^b	Boreal	Y														Y			Y		
<i>Pinus strobus</i> ^{bm}	Boreal		Y							Y			Y	Y	Y	Y					
<i>Pinus sylvestris</i> ^a	Boreal	Y	Y		Y		Y	Y			Y	Y	Y		Y						
<i>Pinus taeda</i> ^{an}	Boreal	Y			Y		Y						Y		Y				Y		
<i>Quercus robur</i> ^{bfi}	Temperate	Y		Y	Y	Y	Y		Y			Y	Y		Y			Y	Y		Y
<i>Robinia pseudoacacia</i> ^{ab}	Temperate	Y																	Y		
Total:	33	25	7	6	24	3	16	8	3	3	6	7	22	4	8	18	3	5	21	4	4

Table B3: Repeated measures ANOVA for CO₂ and CH₄ production.

Factor	F-statistic	d.f.	p	S.E.D
logCO₂				
Species	0.63	1	0.47	1.14
Treatment	54.79	7	<.001	1.10
Treatment*Species	0.81	7	0.59	1.20
Time	1953.21	4	<.001	1.03
Time*Treatment	21.74	28	<.001	1.13
Time*Species	0.17	4	0.89	1.15
Time*Treatment*Species	0.87	28	0.61	1.24
logCH₄				
Species	3.41	1	0.14	1.59
Treatment	5.50	7	<.001	1.29
Treatment*Species	0.15	7	0.99	1.77
Time	206.04	4	<.001	1.13
Time*Treatment	1.23	28	0.28	1.49
Time*Species	5.50	4	0.01	1.63
Time*Treatment*Species	0.29	28	0.99	2.05

Table B4: Two-way ANOVA assessing differences in pH, redox potential and electrical conductivity.

Factor	pH				Redox potential				Conductivity			
	F-statistic	d.f.	p	S.E.D.	F-statistic	d.f.	p	S.E.D.	F-statistic	d.f.	p	S.E.D.
Species	0.07	1,30	0.40	0.09	4.35	1,30	0.05	1.08	2.32	1,30	0.14	1.38
Treatment	53.72	7,30	<.001	0.17	29.38	7,30	<.001	1.16	5.11	7,30	<.001	1.89
Treatment*Species	0.25	7,30	0.89	0.25	0.59	7,30	0.76	1.24	0.57	7,30	0.78	2.47

Table B5: Two-way ANOVA for dehydrogenase, cellulase and xylanase activities. Significance was assessed at $p < 0.05$.

Factor	Dehydrogenase				Cellulase				Xylanase			
	F-statistic	d.f.	p	S.E.D.	F-statistic	d.f.	p	S.E.D.	F-statistic	d.f.	p	S.E.D.
Species	0.67	1,30	0.42	1.10	0.00	1,30	0.98	1.02	0.49	1,30	0.49	1.02
Treatment	22.42	7,30	<.001	1.21	0.25	7,30	0.97	1.03	1.00	7,30	0.45	1.03
Treatment*Species	0.93	7,30	0.50	1.31	0.56	7,30	0.78	1.05	2.19	7,30	0.06	1.04

Appendix C

Table C1: Linear mixed effects models assessing differences CO₂, CH₄ and N₂O fluxes

	F-statistic	d.f.	p	S.E.D
log CO₂				
Treatment	7.06	2,14.8	0.007	0.07
Species	5.18	1,7.7	0.054	0.07
Time	1.06	4,84.1	0.38	0.09
Treatment*Species	0.19	2,14.9	0.829	0.11
Treatment*Time	0.44	8,84.9	0.894	0.15
Species*Time	1.56	4,84.2	0.192	0.13
Treatment*Species*Time	1.6	8,85	0.137	0.22
log CH₄				
Treatment	8.46	2,15.1	0.003	0.2
Species	0.04	1,8	0.854	0.17
Time	6.02	4,65.2	<0.001	0.11
Treatment*Species	0.02	2,15.3	0.985	0.29
Treatment*Time	2.29	8,65.4	0.031	0.24
Species*Time	1.68	4,65.5	0.165	0.19
Treatment*Species*Time	1.94	8,65.7	0.069	0.36
log N₂O				
Treatment	5.59	2,15.4	0.015	0.03
Species	4.9	1,7.7	0.059	0.05
Time	0.27	4,86.6	0.898	0.05
Treatment*Species	1.02	2,15.5	0.385	0.06
Treatment*Time	1	8,87.2	0.443	0.08
Species*Time	2.23	4,86.7	0.072	0.07
Treatment*Species*Time	1.19	8,87.2	0.316	0.12

Table C2: Linear mixed effects models assessing differences in dissolved oxygen with depth

	F-statistic	d.f.	p	S.E.D
Dissolved oxygen				
Depth	142.84	4,96	<0.001	0.02
Treatment	10.48	2,16	0.001	0.02
Species	29.08	1,8	<0.001	0.1
Depth*Treatment	0.26	8,96	0.98	0.04
Depth*Species	3.97	4,96	0.005	0.1
Treatment*Species	3.36	2,16	0.06	0.1
Depth*Treatment*Species	0.22	8,96	0.99	0.1

Table C3: Linear mixed effects models assessing differences in peat physical and chemical properties, and enzyme activities

	Treatment				Species				Treatment*Species			
	F-statistic	d.f.	p	S.E.D	F-statistic	d.f.	p	S.E.D	F-statistic	d.f.	p	S.E.D
Fine roots	17.75	2,16	<0.001	2.0	1.13	1,8	0.319	1.5	0.27	2,16	0.764	2.8
Moisture content	0.15	2,16	0.86	1	1.57	1,8	0.246	1.1	0.71	2,16	0.505	1.5
Organic matter content	1.42	2,16	0.271	1.3	9.83	1,8	0.014	1.6	0.25	2,16	0.782	2.1
pH	3.23	2,16	0.066	0.1	7.43	1,8	0.026	0.1	0.53	2,16	0.6	0.2
Conductivity	0.2	2,16	0.82	22.4	2.05	1,8	0.19	17.1	0.29	2,16	0.75	31.3
Redox	1.14	2,16	0.345	3.9	2.47	1,8	0.155	7.9	0.49	2,16	0.623	7.6
C	6.52	2,16	0.009	1.7	27.65	1,8	<0.001	0.8	3.22	2,16	0.067	2.3
N	3.62	2,16	0.05	0.1	3.1	1,8	0.116	0.1	2.23	2,16	0.14	0.3
C:N	2.59	2,16	0.106	0.5	0.31	1,8	0.594	0.8	0.29	2,16	0.75	0.9
Ammonium	0.75	2,16	0.483	0.6	0.04	1,8	0.846	0.5	0.33	2,16	0.719	0.9
Nitrate	0.79	2,16	0.471	1.5	1.66	1,8	0.234	1.06	0.38	2,16	0.687	2.0
Microbial C	0.38	2,16	0.689	858.5	21.11	1,8	0.002	754.2	0.31	2,16	0.735	1233
DOC	0.84	2,16	0.451	92.7	15.07	1,8	0.005	59.4	1.96	2,16	0.173	125.8
Microbial N	0.51	2,16	0.612	141.5	0	1,8	0.984	103.9	0.73	2,16	0.499	196.2
TDN	0.93	2,16	0.415	352.5	0.07	1,8	0.8	256.2	2.97	2,16	0.08	487.9
Phosphomonoesterase	1	2,16	0.391	0.08	3.6	1,8	0.094	0.1	1.7	2,16	0.214	0.1
Phosphodiesterase	1.25	2,16	0.312	0.07	3.45	1,8	0.1	0.1	1.6	2,16	0.233	0.1
β-glucosidase	0.2	2,16	0.817	0.08	4.53	1,8	0.066	0.1	1.74	2,16	0.207	0.1
N-acetyl-β-glucosaminidase	0.28	2,16	0.761	0.01	2.09	1,8	0.186	0.1	0	2,16	0.998	0.1
Xylanase	0.54	2,16	0.595	0.1	4.69	1,8	0.062	0.2	0.41	2,16	0.67	0.2
Cellobiohydrolase	1.16	2,16	0.34	0.09	8.38	1,8	0.02	0.1	1.72	2,16	0.21	0.2

Table C4: Linear mixed effects models assess differences in peat organic matter properties

	Treatment				Species				Treatment*Species			
	F-statistic	d.f.	p	S.E.D	F-statistic	d.f.	p	S.E.D	F-statistic	d.f.	p	S.E.D
Tpks2	1.8	2,16	0.197	8.6	23.09	1,8	<0.001	8.1	1.01	2,16	0.387	12.5
TOC	3.61	2,16	0.051	2.01	0.15	1,8	0.71	2.3	5.92	2,16	0.012	3.1
HI	4	2,16	0.039	10.3	29.33	1,8	<0.001	8.9	1.47	2,16	0.26	14.7
OI	5.21	2,16	0.018	6.3	0.92	1,8	0.364	5.8	5.65	2,16	0.014	9.2

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