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Root exudate analogues accelerate CO_2 and CH_4 production in tropical peat

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ABSTRACT

Root exudates represent a large and labile carbon input in tropical peatlands, but their contribution to carbon dioxide (CO_2) and methane (CH_4) production remains poorly understood. Changes in species composition and productivity of peatland plant communities in response to global change could alter both inputs of exudates and associated greenhouse gas emissions. We used manipulative laboratory incubations to assess the extent to which root exudate quantity and chemical composition drives greenhouse gas emissions from tropical peatlands. Peat was sampled from beneath canopy palms (*Raphia taedigera*) and broadleaved evergreen trees (*Campnosperma panamensis*) in an ombrotrophic wetland in Panama. Root exudate analogues comprising a mixture of sugars and organic acids were added in solution to peats derived from both species, with CO_2 and CH_4 measured over time. CO_2 and CH_4 production increased under most treatments, but the magnitude and duration of the response depended on the composition of the added labile carbon mixture rather than the quantity of carbon added or the botanical origin of the peat. Treatments containing organic acids increased soil pH and altered other soil properties including redox potential but did not affect the activities of extracellular hydrolytic enzymes. CO_2 but not CH_4 production was found to be linearly related to microbial activity and redox potential. Our findings demonstrate the importance of root exudate composition in regulating greenhouse gas fluxes and propose that *in situ* plant species changes, particularly those associated with land use change, may account for small scale spatial variation in CO_2 and CH_4 fluxes due to species specific root exudate compositions.

1. Introduction

Peatlands are a globally important carbon 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 (CO_2) through plant and soil respiration, and also of methane (CH_4) due to the degradation of organic matter in anoxic conditions. Indeed, wetland environments (including peatlands) contribute 20–39% of global CH_4 emissions and thus play a crucial role in the global carbon cycle (Laanbroek, 2010).

Rates of greenhouse gas 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., 1993). 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., 2016).

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

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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 CO_2 and CH_4 fluxes under both species, with more rapid CO_2 changes dues to preferential use of terminal electron acceptors; ii) increased concentrations of carbon additions will be associated with greater greenhouse gas fluxes; iii) differences in CO_2 and CH_4 fluxes between species will be driven by contrasts in soil biogeochemical properties, and iv) the addition of labile substrates will enhance microbial enzyme activities.

2. Methods

2.1. Study sites

Soil samples were collected in February 2015 from San San Pond Sak, a freshwater and marine wetland located in Bocas del Toro province, Panama. The wetland includes an 80 km^2 ombrotrophic peatland at Changuinola with a central peat dome > 8 m deep that began forming 4000–5000 years ago (Phillips et al., 1997). The peatland features seven distinct plant communities including *Rhizophora mangle* mangrove swamp on the coastal margins, a mixed species mangrove swamp, a *R. taedigera* dominated palm swamp, a mixed species forest swamp, monodominant *C. panamensis* forest swamp, a stunted forest swamp and a *Myrica-Cyrilla* bog-plain (Phillips et al., 1997). This vegetation gradient is matched by a strong decline in nutrient availability towards the interior of the wetland (Sjögersten et al., 2011; Cheesman et al., 2012).

Between 2002 and 2015 mean annual air temperature was 26.3 °C, with little intra-annual variability. During the period of sampling, mean temperature was 25.4 °C. Over the same period, mean annual rainfall was 3207 mm, with a mean of 280 mm in January and February 2015. Mean sub-surface soil temperature is 25.0 °C with limited intra- and inter-annual variation. 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).

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 soil 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.

2.2. Experimental design

2.2.1. Root exudate compound selection

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. 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 CH_4 produced in natural environments has been estimated to originate from methanogenic archaea using acetate as a substrate (Ferry, 1992). Furthermore, formate (alongside CO_2) contributes to the remaining third of CH_4 production, and has been identified in high concentrations bulk forest soils from the southeastern 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 phosphorous-limited peatlands (Sjögersten et al., 2011).

2.2.2. Root exudate compound solution preparation

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 soil 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 (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 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, and prevents lowering soil pH on REC solution addition (Renella et al., 2006). Following preparation, REC solutions were sterilised and stored at 4 °C.

Table 1
Composition and daily addition rates of combined sugar and organic acids in root exudate compound treatments.

Treatment code	Sugar (S) (mg g^{-1} day^{-1})	Organic acids (mg g^{-1} day^{-1})	Total C input (mg g^{-1} day^{-1})
C	0	0	0
S	Fructose - 0.083 Glucose - 0.083 Sucrose - 0.079	0	0.1
SA	Fructose - 0.083 Glucose - 0.083 Sucrose - 0.079	Acetate (A) - 0.171	0.3
SF	Fructose - 0.083 Glucose - 0.083 Sucrose - 0.079	Formate (F) - 0.283	0.3
SM	Fructose - 0.083 Glucose - 0.083 Sucrose - 0.079	Malate (M) - 0.185	0.3
SO	Fructose - 0.083 Glucose - 0.083 Sucrose - 0.079	Oxalate (O) - 0.279	0.3
AFMO	0	Acetate - 0.171 Formate - 0.283 Malate - 0.185 Oxalate - 0.279	0.2
SAFMO	Fructose - 0.083 Glucose - 0.083 Sucrose - 0.079	Acetate - 0.171 Formate - 0.283 Malate - 0.185 Oxalate - 0.279	0.3

2.3. Anoxic assays

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 2 min 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 soil conditions and allow the establishment of the soil 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). CO_2 and CH_4 concentrations were determined using a single injection system, with a 1 ml sample loop that passed the gas sample using N_2 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 H_2 flame ionization (FID) detectors were used to measure CO_2 and CH_4, 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 CO_2 g^{-1} or μg CH_4 g^{-1}, was calculated assuming a linear accumulation rate of gases in the headspace (Hogg et al., 1992).

Table 2
Tree characteristics and sub-surface soil properties. All measures are means ± 1 SE.

Species	*C. panamensis*	*R. taedigera*
Tree height (m)	16.2 ± 0.9	10.4 ± 0.9
DBH (cm)	38.1 ± 2.7	25.0 ± 4.1
Water content (%)	86.3 ± 0.9	77.0 ± 9.5
Organic matter content (%)	90.5 ± 5.3	93.8 ± 1.4
Bulk Density (g cm^{-3})	0.1 ± 0.0	0.1 ± 0.0
pH	5.3 ± 0.3	5.3 ± 0.1
C:N	17.7 ± 1.2	16.8 ± 0.3

2.4. 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 h. Soil organic matter content was determined as the mass lost after ignition for 7 h 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 h. 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) (Table 2). 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.

2.5. 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 μg TPF g^{-1} 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) g^{-1} 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) g^{-1} day^{-1} (Schinner and Vonmersi, 1990).

2.6. 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 soil properties and cumulative CO_2 and CH_4 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 supplementary materials.

3. Results

3.1. CO_2 production

REC addition was associated with a significant increase in CO_2 production ($F_{7,28} = 54.79$, $p < 0.001$) (Fig. 1 a–d) but there was no

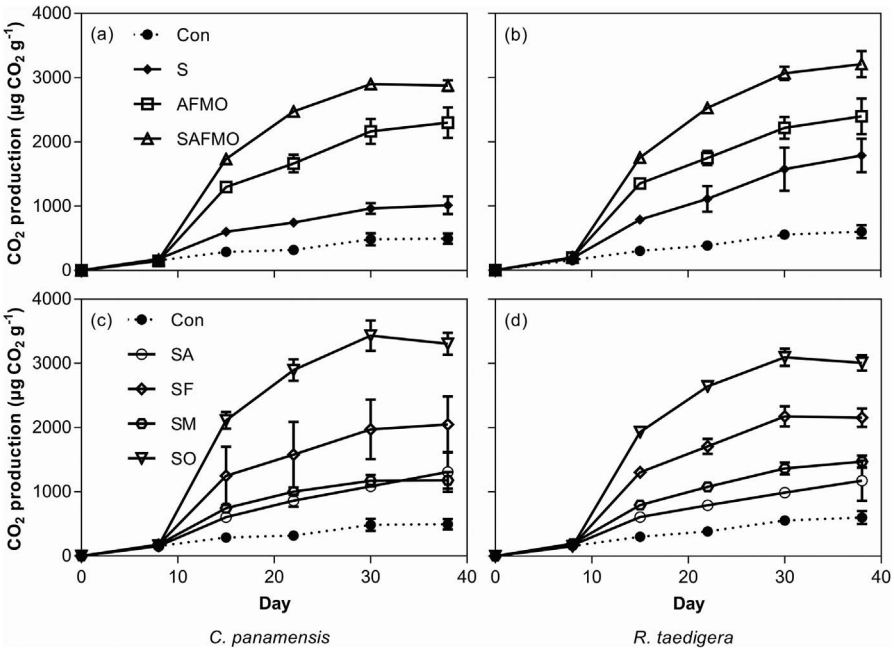

Fig. 1. Cumulative CO_2 efflux from *C. panamensis* (a & c) and *R. taedigera* (b & d) in response to control (Con), sugar (S), combined organic acid (AFMO), combined sugar and organic acid additions (SAFMO) (a & b), and sugar and acetate (SA), sugar and formate (SF), sugar and malate (SM), and sugar and oxalate (SO) additions (c & d). Error bars represent ± 1 SE.

difference in CO_2 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 independent of species. There was no significant interaction between time following incubation, treatment and species ($F_{28,128}$ = 0.87, p = 0.61).

3.2. CH_4 production

REC addition significantly increased CH_4 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) (Fig. 2 a–d). Immediately following the end of treatment, CH_4 production from sugars was greater than other treatments but by day 38 cumulative CH_4 production was greater in the combined sugar and organic acid treatments. In contrast to changes in CO_2 production, not all treatments increased CH_4 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 CH_4 fluxes compared to the control. For *C. panamensis*, sugar and formate addition also reduced CH_4 fluxes by 26.6% compared to the control.

3.3. Soil properties and enzyme assays

The majority of REC treatments were associated with significant changes in soil properties, but there were limited differences between species. With the exception of sugar, and sugar and malate treatments, all REC additions significantly increased pH ($F_{7,30}$ = 53.72, p < 0.001) (Fig. 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

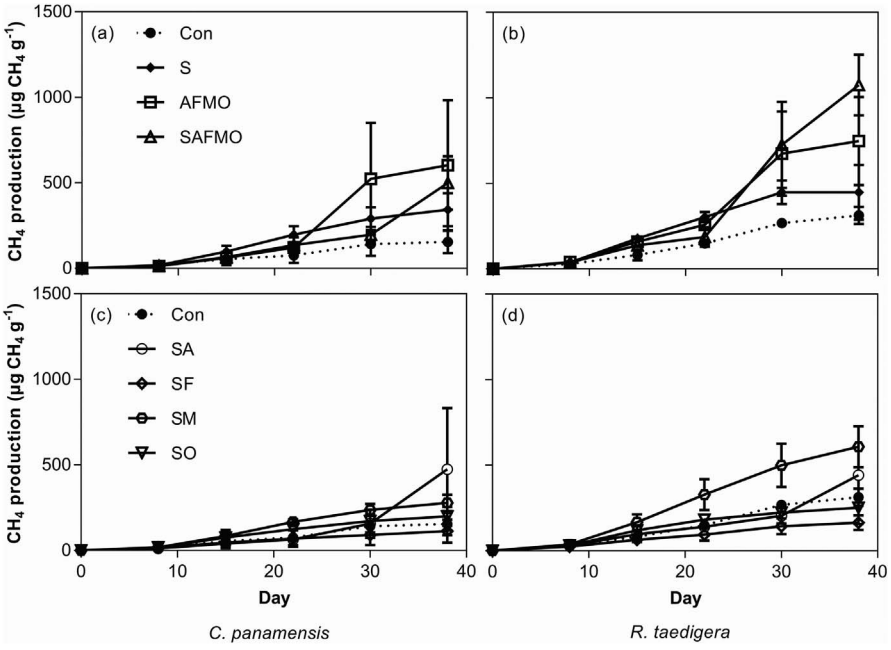

Fig. 2. Cumulative CH_4 efflux from *C. panamensis* (a & c) and *R. taedigera* (b & d) in response to control (Con), sugar (S), combined organic acid (AFMO), combined sugar and organic acid additions (SAFMO) (a & b), and sugar and acetate (SA), sugar and formate (SF), sugar and malate (SM), and sugar and oxalate (SO) additions (c & d). Error bars represent ± 1 SE.

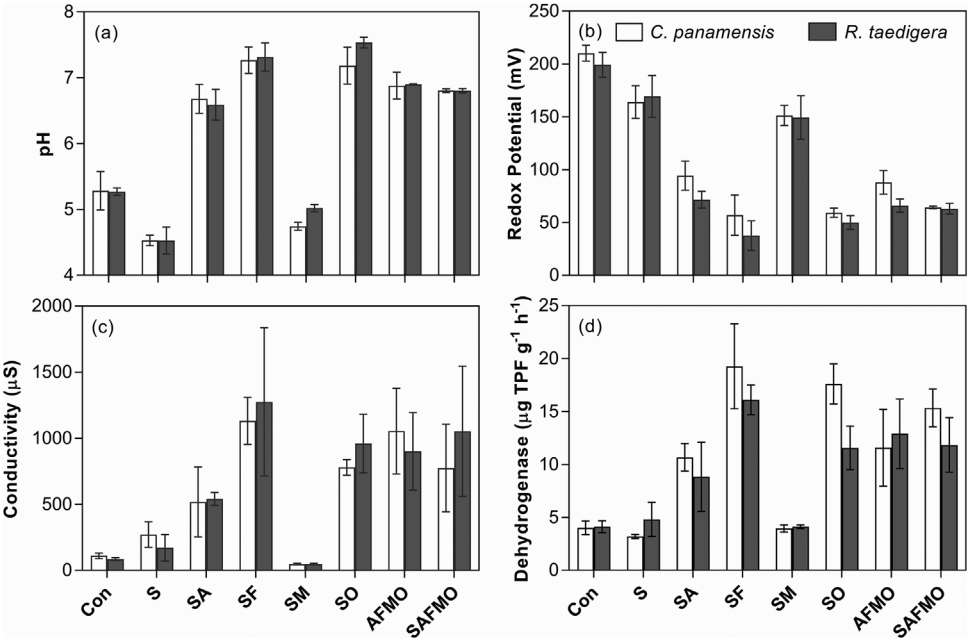

Fig. 3. (a) pH, (b) redox potential, (c) electrical conductivity, and (d) dehydrogenase activity of *C. panamensis* and *R. taedigera* peats following treatment and 38 days incubation. Error bars represent ± 1 SE.

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) (Fig. 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) (Fig. 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).

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 g^{-1} h^{-1}) and *R. taedigera* (16.1 mg TPF g^{-1} h^{-1}) and lowest activity occurred for sugar treatment for *C. panamensis* (3.2 mg TPF g^{-1} h^{-1}) and for sugar and malate for *R. taedigera* (4.1 mg TPF g^{-1} h^{-1}) (Fig. 3d). In contrast to dehydrogenase activity, there was no significant difference in cellulase or xylanase activities on REC addition (see supplementary materials).

3.4. Linking soil properties and CO_2 and CH_4 production

No significant relationships were identified between cumulative CH_4 production and any measured soil properties. However, a significant relationship was found between cumulative CO_2 production and changes in redox potential ($T_{2,45}$ = 8.04, p = 0.01) (Fig. 4a) and dehydrogenase activity ($T_{2,45}$ = 2.84, p = 0.05) (Fig. 4b). In both instances, control and sugar treatments cluster together, with individual and combined organic acid additions grouped together.

4. Discussion

4.1. Species influences on CO_2 and CH_4 production

The addition of labile substrates significantly increased both CO_2 and CH_4 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 CO_2 and CH_4 fluxes were greater from *R. taedigera* than *C. panamensis*, with strong positive relationships demonstrated between the availability of labile carbon and production. Differences in soil 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., 2016).

Additions of sugars, amino and organic acids have previously been found to elicit different responses from forest and agricultural soils, with changes in CO_2 production more pronounced in soils containing soil 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., 2016), rates of decomposition (Hoyos-Santillan et al., 2015) and CO_2 and CH_4 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., 2016). 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 CO_2 and CH_4 fluxes alongside differences in organic matter properties and litter properties (Hoyos-Santillan et al., 2015, 2016).

4.2. CO_2 and CH_4 response rates

Increases in CO_2 production occurred more rapidly than changes in CH_4 production during incubation, resulting in relatively high CO_2:CH_4 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, H_2 and CO_2. These products are subsequently oxidised in conjunction with the reduction of inorganic terminal electron acceptors such as nitrate (NO_3^-) and sulphate (SO_4^{2-}), and organic acceptors such as humic acids (Lovley et al., 1996; Lipson et al., 2010). These electron acceptors are used preferentially,

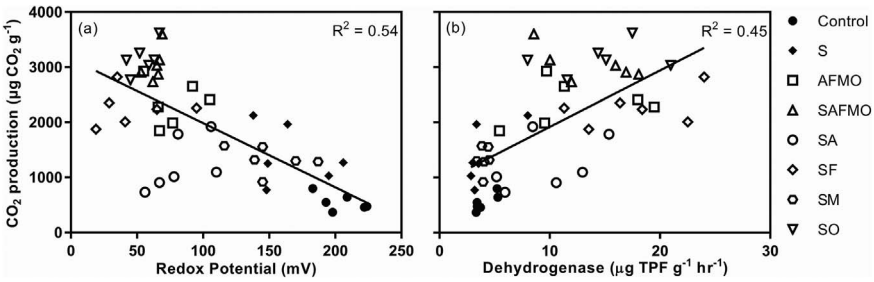

Fig. 4. Linear regression linking changes in cumulative CO_2 evolved to changes in (a) redox potential and (b) dehydrogenase activity.

driving CO_2 production, before CH_4 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 CO_2 and CH_4 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 CO_2 and CH_4 fluxes.

Sugar and formate, and sugar and oxalate additions were found to have an inhibitory effect on CH_4 production but not on CO_2 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 soil pH (Fig. 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.

4.3. Soil properties and CO_2 and CH_4 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 CO_2 production indicates the link between of microbial activity and CO_2 derived from microbial respiration. Higher rates of microbial activity, stimulated by labile carbon, are therefore associated with increased respiration and CO_2 production (Fig. 4b).

Sugar addition alone did not significantly enhance dehydrogenase activity compared to the control, despite being associated with elevated CO_2 and CH_4 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 CO_2 and CH_4 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 (Fig. 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 soil electrical conductivity for most treatments due to changes in the availability of nutrients and salts within the soils.

REC solutions did not significantly increase xylanase or cellulase activities (see supplementary materials) 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 CH_4 production. This suggests that while changes in CO_2 flux are partially influenced by changes in soil properties, particularly redox potential, CH_4 production is regulated by the accessibility of substrates for methanogens and the availability of terminal electron acceptors.

4.4. Conclusion

This study shows that inputs of labile root exudate analogues are able to drive strong increases in CO_2 and CH_4 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 utilized 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 CO_2 but not CH_4 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 CO_2 and CH_4 fluxes.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2017.11.008.

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