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ROOT EXUDATES AND CARBON EMISSIONS FROM TROPICAL PEATLANDS

Nicholas T. Girkin[1], Nick Ostle[2], Benjamin L. Turner[3] and Sofie Sjogersten[1]

[1]*School of Biosciences, University of Nottingham, Nottingham, UK*
[2]*Lancaster Environment Centre, Lancaster University, Lancaster UK*
[3]*Smithsonian Tropical Research Institute, Ancon, Republic of Panama*

SUMMARY

Plant root exudates represent a large, labile carbon (C) input to tropical peatlands, but their contribution to methane (CH_4) and carbon dioxide (CO_2) emissions remain poorly understood. Peat under two contrasting vegetation types, the palm *Raphia taedigera* and the broadleaf evergreen tree *Campnosperma panamensis*, from a coastal peatland in Panama, were incubated anaerobically and received daily C additions for two weeks. C was added as a combination of three sugars or four organic acids, with the effects on CH_4 and CO_2 production measured over 40 days. C additions significantly increased CH_4 and CO_2 production under both vegetation types, although emissions were significantly greater under palms. Addition of organic acids was also associated with increases in pH and dehydrogenase activity, likely due to changes in microbial populations. Taken together, these results indicate that regular additions of root exudates are associated with increased fluxes and microbial activity, depending on composition, may enhance C mineralisation.

Keywords: *Methane, root exudates, tropical, wetland*

INTRODUCTION

Tropical forested peatlands are a globally important C store that is under significant threat from climate and land use changes (Page *et al*., 2011). While it is known that CH_4 and CO_2 fluxes can differ significantly between different vegetation types (Wright *et al*., 2013), the mechanisms responsible for these differences remain poorly understood.

Plants can release significant quantities of photosynthetically derived C into the rhizosphere as root exudates, which play an important role in regulating plant–microbial interactions (Shi *et al*., 2011). Exudates consist primarily of sugars, organic acids, and amino acids (Walker *et al*., 2003). Root exudates contain low concentrations of C compared to inputs from decaying plant material, but their labile nature means that they are rapidly utilised by the microbial community (Kuzyakov and Domanski, 2000).

While sugars are generally considered to be the dominant exudate in grasslands and agricultural systems, low molecular weight organic acids are more significant components of forest soil profiles, often present at two to three times the concentration of sugars (Smith, 1976, Grayston and Campbell, 1996, Shi *et al*., 2011). However, 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). Few studies have examined tropical forests or peatlands.

We carried out an anaerobic incubation study of tropical peats from two contrasting plant successional communities dominated by the palm *R. taedigera* and a broadleaf evergreen tree, *C. panamensis.* After two weeks incubation at 30°C, peats were treated with a combination of three sugars (glucose, fructose and sucrose) and four organic acids (acetic acid, formic acid, malic acid and oxalic acid) for two weeks. We quantified total CH_4 and CO_2 production and changes to pH and microbial dehydrogenase activity.

METHODS

Subsurface peat samples were collected from Bahia Almirante (09° 18‘ 13.00”N, 82° 21‘ 13.80”W) from a mixed forest stand located 600 m from the coast. Samples were collected under three *C. panamensis* trees and three *R. taedigera* palms within 0.5 m of the nearest tree. 7.5g of dry weight equivalent soil were water saturated and sealed in 48 replicate 120 mL glass serum bottles after flushing with N_2 to generate anaerobic conditions. Microcosms were incubated at 30°C for two weeks prior to the first addition of root exudate compound solutions to allow the establishment of the microbial communities.

In the absence of root exudate profiles for *R. taedigera* and *C. panamensis*, the selection of substrates was based on several criteria. Across all ecosystems fructose, glucose and sucrose have consistently been identified as

the most abundant sugars in root exudates (Smith, 1976, Jones, 1998, Shi *et al*., 2011) Acetic acid is the most important substrate for methanogenesis in peatlands (Ferry, 1992, Lloyd *et al*., 1998). Previous incubation experiments have highlighted that malic acid can have a significant priming effect on CO_2 fluxes (Chowdhury *et al*., 2014). Furthermore, malic acid, alongside oxalic acid, was amongst the most commonly occurring organic acids in the root exudates of several forest species (Smith, 1976; Grayston and Campbell, 1996, Jones, 1998, Strobel, 2001). Formic acid has previously been detected in high concentrations in forest soils (Fox and Comerford, 1990) and is also an additional substrate for methanogenesis in peat (Ferry, 1992).

Sugars and organic acids were added in combination over a 14 day period at concentrations of 0.1 mg, 0.2 mg, and 0.3 mg C g^{-1} day^{-1} dissolved in 1 mL deionised water. Additions at these concentrations closely match rates reported previously and mimic the gradual release of exudates over time (Baudoin *et al*., 2003, Henry *et al*., 2008).

Sugar additions consisted of a C input of 0.1 mg g^{-1} dry soil day^{-1} of equal quantities of fructose, glucose and sucrose. Organic acid additions (AFMO) comprised a C input of 0.2 mg g^{-1} dry soil day^{-1} of acetic, formic, malic and oxalic acids. Combined sugars and organic acids (SAFMO), sugar and acetic acid (SA), sugar and formic acid (SF), sugar and malic acid (SM), and sugar and oxalic acid (SO) were added at a C input of 0.3 mg g^{-1} dry soil day^{-1}, with sugars comprising 0.1 mg and organic acids comprising 0.2 mg. A 2:1 ratio of organic acids to sugars matched reported ratios in forest exudate profiles (Smith, 1976, Grayston and Campbell, 1996, Shi *et al*., 2011).

C additions began 14 days after the preparation of the microcosms to establish background rates. Headspace gas samples were collected weekly and analysed by gas chromatography. The potential rate of gas production, expressed as µg CO_2 g^{-1} or µg CH_4 g^{-1}, was calculated assuming the linear accumulation of gases in the headspace over time (Hogg *et al*., 1992).

Soil pH and dehydrogenase activity were measured after 14 days (prior to treatment) and after 40 days at the conclusion of the experiment. Dehydrogenase activity, one of the principle components of soil enzymatic activities was assessed using the reduction of 2,3,5-triphenyltetrazolium chloride to triphenylformazan (Ohlinger, 1995) and was used as an indicator of microbial populations and activity. Analysis of variance was performed using the Residual Maximum Likelihood method (REML) in GenStat (v15.1.). Rates of CH_4 and CO_2 were logged to comply with the requirements of REML. Differences in dehydrogenase activity and pH were analysed by two-way ANOVA.

RESULTS

For both *R. taedigera* and *C. panamensis*, the addition of sugars and organic acids significantly enhanced CH_4 production ($p < 0.05$) (Figure 1A). The greatest cumulative CH_4 fluxes for *R. taedigera* were recorded under SAFMO treatment with an increase of 243.8% to 1075.1 µg CH_4 g^{-1} compared to the control.In contrast under *C. panamensis* the maximum measured CH_4 fluxes were recorded under AFMO treatment, an increase of 139.24% to 748.2 µg CH_4 g^{-1} compared to the control. SO and SF treatments showed a decrease in CH_4 flux of 19.8% and -47.9% under *R. taedigera*, but under *C. panamensis* only SF caused an inhibitory effect (-26.6%). Only the inhibitory effect from the SF treatment was found to be significant ($p > 0.05$). There was a significant difference in cumulative CH_4 fluxes between *R. taedigera* and *C. panamensis*, with mean fluxes higher under *R. taedigera* ($p < 0.05$).

All sugar and organic acid additions significantly enhanced CO_2 compared to the control ($p < 0.05$) (Figure 1B). The greatest flux for *R. taedigera* was associated with SAFMO addition, with an increase of 434.4% to 3212.7 µg CO_2 g^{-1}. For *C. panamensis* the greatest flux was recorded for SO addition with a 566.3% increase to 3304.8 µg CO_2 g^{-1}. There was no significant difference between these treatments. In contrast to results for CH_4 fluxes, SF treatments significantly enhanced CO_2 fluxes compared to the control. There was a significant difference in cumulative CO_2 fluxes between *R. taedigera* and *C. panamensis*, with mean fluxes higher under *R. taedigera* ($p < 0.05$).

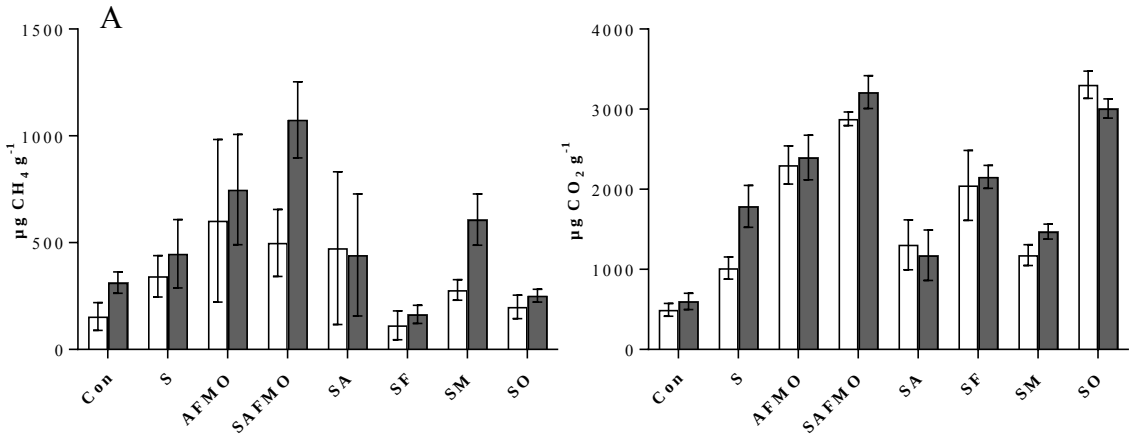

Figure 1: A.Total CH_4 production for *C. panamensis* (white) and *R. taedigera* (grey) after 40 days. B. Total CO_2 production for *C. panamensis* (white) and *R. taedigera* (grey) after 40 days. Con = Control, S = sugars, AFMO = organic acids, SAFMO = sugars + organic acids, SA = sugars + acetic acid, SF = sugars + formic acid, SM = sugars + malic acid, SO = sugars + oxalic acid. Error bars equal 1 SEM.

There was no significant difference in peat dehydrogenase activity after 14 days, prior to root exudate addition ($p > 0.05$). At the conclusion of the experiment sugar and SM additions did not significantly increase dehydrogenase activity compared to the control ($p > 0.05$). All other treatments significantly enhanced dehydrogenase activity ($p < 0.05$) (Figure 2A). There was no significant difference in dehydrogenase activity between *R. taedigera* and *C. panamensis*, although mean activity was greatest under *R. taedigera* ($p > 0.05$). Greatest dehydrogenase activity was measured under SF for both *R. taedigera* (1345.0 mg TPF g^{-1} h^{-1}) and *C. panamensis* (1097.0 mg TPF g^{-1} h^{-1}).

All additions affected soil pH: sugar addition significantly decreased pH from 5.3 to 4.5 ($p < 0.05$) (Figure 2B). SM additions also lowered pH to 4.9 but not significantly ($p < 0.05$). All other treatments significantly increased pH, with the greatest increase occurring under SO additions, to pH 7.4 ($p < 0.05$). Soil pH was greater under *R. taedigera* but did not differ significantly from *C. panamensis* ($p > 0.05$).

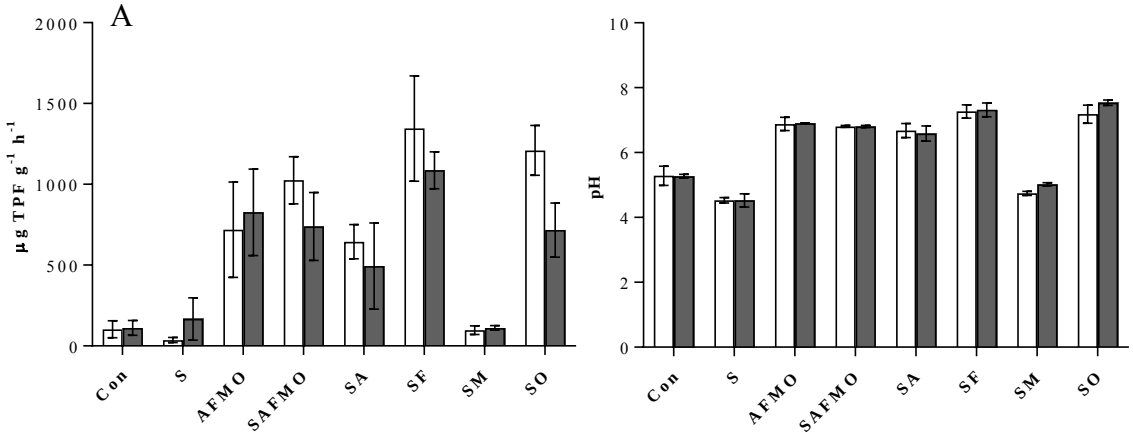

Figure 2: A. Dehydrogenase activity, B. pH. *C. panamensis* (white) and *R. taedigera* (grey) after 40 days. Con = Control, S = sugars, AFMO = organic acids, SAFMO = sugars + organic acids, SA = sugars + acetic acid, SF = sugars + formic acid, SM = sugars + malic acid, SO = sugars + oxalic acid. Error bars equal 1 SEM.

DISCUSSION

Differences in *in situ* surface CH_4 and CO_2 fluxes between *R. taedigera* and *C. panamensis* may be associated with the different decomposition rates previously reported for peats formed from both species (Wright *et al*., 2013). *R. taedigera* surface litter decomposes more rapidly than *C. panamensis* litter, although the effect varies with depth and plant tissue (Hoyos-Santillan *et al*., 2015). The extent of the response differed between peat types: the same addition of 0.1 mg C per day of sugars elicited a greater increase in CH_4 production from *C. panamensis* of 122.3% compared to 43.0% for *R. taedigera*. Responses of this nature have previously been reported when labile C was added to bulk soil and the rhizosphere, with differences ascribed to differing bacterial communities (Baudoin *et al.*, 2003).

Despite being identified as the most significant substrate for methanogenesis in peatlands, after 40 days CH_4 and CO_2 production under SA addition did not differ significantly from sugar addition alone, despite the total C

input being higher (0.3 mg C g^{-1} day^{-1} compared to 0.1 mg C g^{-1} day^{-1}). Previously the addition of acetic acid alone has been found to trigger greater peat mineralisation than the addition of the same concentration of glucose or fructose (Hamer and Marschner, 2002).

SF and SO additions were found to have an inhibitory effect only on CH_4 production but not on CO_2 production. In the case of SO, this effect was only observed for *R. taedigera*. Organic acid additions have previously been reported to have an inhibitory effect, with negative responses observed in changes to bacterial taxa diversity and abundance (Shi *et al*., 2011). This effect may be driven by the direct inhibition of microbial activity through the presence of specific organic acids. Alternatively the SA, SF and SO responses may arise through a competition effect, with taxa that utilise organic acids as a C source being outcompeted by other more rapidly growing microorganisms that are better able to respond to changes in environmental conditions, such as the increase in soil pH (Figure 2b) (Paterson *et al*., 2007).

Differences in hydrolytic enzymatic activity have previously been reported along a plant successional gradient that included *R. taedigera* and *C. panamensis* in Bocas del Toro (Sjogersten, 2010) but no significant difference were observed on this occasion between species. Sugar addition did not significantly increase dehydrogenase activity compared to the control despite being associated with significant increases in CH_4 and CO_2 production. This may be because sugars are more readily utilised than organic acids and thus their effects are seen more quickly, with enzyme activity only being measured after the activity in sugar treated peats had declined: in an arable ecosystem, sugar and amino acid amendments triggered the activation of the soil microbial communities in less than a minute (Jones and Murphy, 2007). It is likely that sugar treatments induced fewer changes in the microbial community than organic acids as the latter stimulated a reduced group of specialised microorganisms (Landi *et al*., 2006). Sugar additions have previously been found to increase dehydrogenase activity when added at the same concentration (Shi *et al*., 2011), although this difference may be because activity was measured two weeks after the last addition of root exudates by which time changes in dehydrogenase activity stimulated by sugars may have declined.

All organic acid additions, with the exception of SM, increased pH significantly compared to the control. The lower dehydrogenase activity recorded for SM may be associated with the lower pH (Trevors, 1984). 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 CH_4 (Wang *et al*., 1993) and CO_2 production (Yan *et al*., 1996). However, pH and dehydrogenase activity were not found to be significant determining factors for CH_4 and CO_2 production ($p > 0.05$).

CONCLUSIONS

Peats from contrasting vegetation types differ significantly in their responses to labile C addition. Greater increases in CH_4 and CO_2 fluxes were measured from soils under *R. taedigera* compared to *C. panamensis*. These differences are of significance due to the continued conversion of many peatlands to oil pal plantations. This is likely to be because of the different composition of the peat types and differences in the initial microbial communities (Hoyos-Santillan *et al*., 2015). While low C concentration sugar additions were associated with greater CH_4 and CO_2 fluxes than some higher C organic acid treatments, enzyme activity and pH were generally higher under organic acid addition. This is likely to be associated with longer term changes in microbial composition and may lead to later increases in fluxes through increased C mineralisation.

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