



Large impacts of small methane fluxes on carbon isotope values of soil respiration

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ABSTRACT

Carbon dioxide isotope ($\delta^{13}\text{C}$ of CO_2) analysis is increasingly used to address a broad range of questions involving soil C dynamics and respiration sources. However, attaining $\delta^{13}\text{C}$ mass balance is critical for robust interpretation. Many ecosystems exhibit methane (CH_4) fluxes that are small in the context of total C budgets, yet may significantly impact $\delta^{13}\text{C}$ values of CO_2 due to large kinetic fractionations during CH_4 production. Thus, the $\delta^{13}\text{C}$ values of CO_2 do not directly reflect respiration C sources when co-occurring with CH_4 , but few studies of terrestrial soils have considered this phenomenon. To assess how CH_4 altered the interpretation of $\delta^{13}\text{C}$ values of CO_2 , we incubated a Mollisol and Oxisol amended with C_4 -derived plant litter for 90 days under two head-space treatments: a fluctuating anaerobic/aerobic treatment (four days of anaerobic conditions alternating with four days of aerobic conditions), and a static aerobic treatment (control). We measured $\delta^{13}\text{C}$ values of CO_2 and CH_4 with a tunable diode laser absorption spectrometer, using a novel in-line combustion method for CH_4 . Cumulative $\delta^{13}\text{C}$ of CO_2 differed significantly between treatments in both soils. The $\delta^{13}\text{C}$ values of CO_2 were affected by relatively small CH_4 fluxes in the fluctuating anaerobic/aerobic treatment. Effects of CH_4 on $\delta^{13}\text{C}$ values of CO_2 were greater in the Oxisol due to its higher percent contribution of CH_4 to total C mineralization (18%) than in the Mollisol (3%) during periods of elevated CH_4 production. When CH_4 accounted for just 2% of total C mineralization, the $\delta^{13}\text{C}$ values of CO_2 differed from total C mineralization by 0.3–1‰, and by 1.4–4.8‰ when CH_4 was 10% of C mineralization. These differences are highly significant when interpreting natural abundance $\delta^{13}\text{C}$ data. Small CH_4 fluxes may strongly alter the $\delta^{13}\text{C}$ values of CO_2 relative to total mineralized C. A broad range of mineral and peatland soils can experience temporary oxygen deficits. In these dynamic redox environments, the $\delta^{13}\text{C}$ values of CO_2 should be interpreted with caution and ideally combined with $\delta^{13}\text{C}$ of CH_4 when partitioning sources and mechanisms of soil respiration.

1. Introduction

Over the recent decades, stable carbon isotope ($\delta^{13}\text{C}$) analyses have been extensively used to understand belowground C processes, especially to quantify the sources and dynamics of soil carbon dioxide (CO_2) emissions (Amundson et al., 1998; Ehleringer et al., 2000). For instance, measurements of $\delta^{13}\text{C}$ of CO_2 at natural abundance and in ^{13}C labeling experiments can enable partitioning of heterotrophic and autotrophic respiration (Hanson et al., 2000; Tu and Dawson, 2005), quantification of turnover rates for different soil organic C pools (Collins et al., 2000; Vestergård et al., 2016), and identification of biogeophysical processes influencing gas dynamics in the soil system (Moyes et al., 2010; Bowling et al., 2015). Robust interpretation of $\delta^{13}\text{C}$ values of soil respiration is thus important for our understanding of soil and ecosystem C dynamics.

The $\delta^{13}\text{C}$ values of soil respiration are often thought to reflect $\delta^{13}\text{C}$

of the substrate from which the CO_2 was derived (Ehleringer et al., 2000; Breecker et al., 2015; Hall et al., 2017). However, production of methane (CH_4) impacts the interpretation of $\delta^{13}\text{C}$ values of CO_2 . When CO_2 co-occurs with methane (CH_4), the $\delta^{13}\text{C}$ values of the net CO_2 flux may be affected by C isotope fractionation during both methanogenesis and CH_4 oxidation (Fig. 1). The fractionation factor (ϵ) for CH_4 production is defined here as: $\epsilon = ((1000 + \delta^{13}\text{C}_C)/(1000 + \delta^{13}\text{C}_{\text{CH}_4}) - 1) \times 1000 \approx \delta^{13}\text{C}_C - \delta^{13}\text{C}_{\text{CH}_4}$ (Hayes, 1993), where $\delta^{13}\text{C}_C$ and $\delta^{13}\text{C}_{\text{CH}_4}$ are $\delta^{13}\text{C}$ values of the C source (either CO_2 or acetate) and CH_4 , respectively. During methanogenesis, both the hydrogenotrophic (CO_2 reduction; $\text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$) and acetoclastic (acetate fermentation; $\text{CH}_3\text{COOH} \rightarrow \text{CH}_4 + \text{CO}_2$) pathways impose large fractionations, with ϵ values of 30–90‰ and 7–35‰, respectively (Penning et al., 2005; Conrad and Claus, 2009; Blaser and Conrad, 2016). These result in much lower $\delta^{13}\text{C}$ values in CH_4 relative to the C substrate (either CO_2 or acetate). By mass balance, residual CO_2 from

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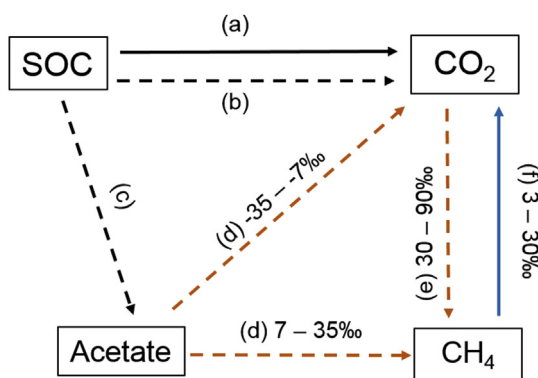


Fig. 1. Schematic of processes affecting C isotope ratios ($\delta^{13}\text{C}$ values) of CH_4 and CO_2 in fluctuating anaerobic/aerobic soils. The numbers on the lines indicate C isotope fractionation factors (ϵ). The different letters on the lines indicate different processes: Production of CO_2 from soil respiration (a, b); soil organic C fermentation to acetate (c); acetoclastic methanogenesis (d); hydrogenotrophic methanogenesis (e); CH_4 oxidation (f). The solid line indicates aerobic conditions, and the dashed line indicates anaerobic conditions. The lines in black denote minor C fractionation; the lines in orange indicate that fractionation increased $\delta^{13}\text{C}$ values of CO_2 ; and the line in blue indicates that fractionation decreased $\delta^{13}\text{C}$ values of CO_2 . (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the hydrogenotrophic pathway and CO_2 produced by acetate fermentation must be enriched in ^{13}C to balance the more depleted ^{13}C of CH_4 (Whiticar, 1999; Hornibrook et al., 2000). Conversely, CH_4 consumption by aerobic (and potentially anaerobic) oxidation preferentially removes isotopically lighter C ($\epsilon = 3\text{--}30\text{‰}$; Happell et al., 1994), resulting in higher $\delta^{13}\text{C}$ values of CH_4 and lower $\delta^{13}\text{C}$ values of CO_2 (Fig. 1). Hence, it is clearly important to consider CH_4 fractionation effects on $\delta^{13}\text{C}$ values of CO_2 . These processes have been reasonably well documented in studies of traditional wetland ecosystems (i.e., consistently saturated soils). For example, previous studies have observed more positive $\delta^{13}\text{C}$ values of CO_2 than bulk soil $\delta^{13}\text{C}$ in peatlands as a consequence of CH_4 production (Corbett et al., 2013; Holmes et al., 2015).

It remains uncertain, however, whether the influence of CH_4 on $\delta^{13}\text{C}$ values of CO_2 is also important in terrestrial soils that experience only sporadic or spatially limited O_2 deprivation, and correspondingly small net CH_4 emissions. According to isotope mass balance, the $\delta^{13}\text{C}$ value of total mineralized C ($\text{CO}_2 + \text{CH}_4$) can be calculated as: $\delta^{13}\text{C}_{\text{TC}} = P_{\text{CH}_4}/100 \times \delta^{13}\text{C}_{\text{CH}_4} + (1 - P_{\text{CH}_4}/100) \times \delta^{13}\text{C}_{\text{CO}_2}$, where P_{CH_4} is the percentage of CH_4 to total mineralized C (hereafter denoted “ CH_4 percentage”). This equation can be expressed as $\delta^{13}\text{C}_{\text{TC}} - \delta^{13}\text{C}_{\text{CO}_2} = -P_{\text{CH}_4}/100 \times (\delta^{13}\text{C}_{\text{CO}_2} - \delta^{13}\text{C}_{\text{CH}_4})$ to reflect the impact of CH_4 on the $\delta^{13}\text{C}$ value of CO_2 ($\epsilon_{\text{TC-CO}_2}$). According to the above fractionation factors, the difference between $\delta^{13}\text{C}$ values of CO_2 and CH_4 is expected to vary from 7 to 90‰ in soils under anaerobic conditions, in which hydrogenotrophic and/or acetoclastic methanogenesis occur without any CH_4 oxidation. However, few studies explored C isotope separation between CO_2 and CH_4 ($\delta^{13}\text{C}_{\text{CO}_2} - \delta^{13}\text{C}_{\text{CH}_4}$) in soils with sporadic temporal or spatial O_2 limitation. Preliminary calculations suggest that small CH_4 fluxes could significantly impact the interpretation of $\delta^{13}\text{C}$ values of CO_2 . If we assume a difference in $\delta^{13}\text{C}$ values of CH_4 and CO_2 of 30‰, a 5% contribution of CH_4 to total mineralized C would result in $\delta^{13}\text{C}$ values of CO_2 that are 1.5‰ greater than total mineralized C. This difference would often be highly significant in the context of ecosystem $\delta^{13}\text{C}$ budgets, where differences $< 1\text{‰}$ can provide insights about local and global C cycle processes (Bowling et al., 2014).

Methane production is typically thought to occur under highly reducing conditions that are most prevalent in wetlands or aquatic

sediments (Conrad, 1996). However, terrestrial soils can also have low O_2 concentrations in microsites resulting from imbalances in biological O_2 consumption relative to diffusive re-supply (Sexstone et al., 1985). Fluctuations in soil O_2 availability following rain, irrigation, snowmelt, and/or soil frost occur across a broad range of ecosystems including humid forests, grasslands, urban lawns, and croplands (Liptzin et al., 2011; Hall et al., 2013, 2016; Moyes and Bowling, 2013; Jarecke et al., 2016; O’Connell et al., 2018). Temporary depletion of O_2 and other terminal electron acceptors can provide favorable conditions for methanogenesis, and both gross and net CH_4 production have been shown to occur even in bulk aerobic soils (von Fischer and Hedin, 2007; Liptzin et al., 2011; Yang and Silver, 2016). This implies that it may frequently be necessary to account for trace CH_4 production and its $\delta^{13}\text{C}$ values when using $\delta^{13}\text{C}$ of CO_2 to understand C cycling processes. However, co-occurring measurements of $\delta^{13}\text{C}$ of CO_2 and CH_4 from uplands (and even some wetland ecosystems, such as arctic peatlands) remain relatively uncommon.

Here, we incubated a temperate Mollisol and a tropical Oxisol under a fluctuating anaerobic/aerobic condition over 90 days to simulate redox fluctuations driven by variations in moisture and C supply that occur in their natural ecosystem contexts, along with a static aerobic condition (control). We assessed the effects of the fluctuating anaerobic/aerobic treatment on $\delta^{13}\text{C}$ values of CO_2 , CH_4 and total mineralized C ($\text{CO}_2 + \text{CH}_4$). We hypothesized that the fluctuating anaerobic/aerobic treatment would alter $\delta^{13}\text{C}$ values of CO_2 relative to $\delta^{13}\text{C}$ of soil mineralized C to a significant extent for ecological interpretation (i.e., one – several ‰) when relatively small CH_4 fluxes ($P_{\text{CH}_4} \sim 5\%$) occurred.

2. Materials and methods

2.1. Soil sampling

We sampled a Mollisol and Oxisol characterized by redox fluctuations in March 2017. The Mollisol was from a topographic depression in a field under corn-soybean cultivation in north-central Iowa (41°75’N, 93°41’W), USA, and the Oxisol was from an upland valley in a perhumid tropical forest near the El Verde field station of the Luquillo Experimental Forest (18°17’N, 65°47’W), Puerto Rico. The Mollisol was formed from till following the Wisconsin glaciation and developed under tallgrass prairie and wetland vegetation. The depression has very poorly drained soils described as mucky silt loam (fine, montmorillonitic, mesic Cumulic Haplaquoll) that experience periodic flooding (Logsdon, 2015). This site was cultivated with corn (*Zea mays*) and soybean (*Glycine max*) rotated on an annual basis. The Mollisol was sampled following a corn cultivation phase. We collected soils from the plow layer A horizon (0–20 cm), which is mixed via tillage or cultivation every year. Six soil cores (10.2 cm diameter) were sampled in a 50 × 50-m region, and then composited to generate spatially representative samples. The Oxisol was formed from volcanoclastic sediments (Buss et al., 2017). This soil experiences temporal shifts in bulk O_2 concentrations, varying from 0% to 21% O_2 over scales of hours to weeks (Liptzin et al., 2011). Six replicate soil cores were sampled from the A horizon (0–10 cm) of the valley site, composited, and shipped overnight to Iowa State University. We chose to assay the surface A horizons from both soils, given that their rates of anaerobic biogeochemical activity at the surface were higher than in deeper horizons due to greater C availability (Hall et al., 2014; Huang and Hall, 2017).

2.2. Optical $\delta^{13}\text{C}$ analysis method

The $\delta^{13}\text{C}$ values of CO_2 and CH_4 are traditionally measured by continuous flow-isotope ratio mass spectrometry. However, relatively low sample throughput and high costs potentially limit measurement frequency and the capacity to capture temporal variation at short time scales (e.g., hourly – daily) relevant to $\delta^{13}\text{C}$ dynamics over prolonged

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