9-2018

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

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Abstract
Carbon dioxide isotope (δ13C of CO2) analysis is increasingly used to address a broad range of questions involving soil C dynamics and respiration sources. However, attaining δ13C mass balance is critical for robust interpretation. Many ecosystems exhibit methane (CH4) fluxes that are small in the context of total C budgets, yet may significantly impact δ13C values of CO2 due to large kinetic fractionations during CH4 production. Thus, the δ13C values of CO2 do not directly reflect respiration C sources when co-occurring with CH4, but few studies of terrestrial soils have considered this phenomenon. To assess how CH4 altered the interpretation of δ13C values of CO2, we incubated a Mollisol and Oxisol amended with C4-derived plant litter for 90 days under two headspace 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 δ13C values of CO2 and CH4 with a tunable diode laser absorption spectrometer, using a novel in-line combustion method for CH4. Cumulative δ13C of CO2 differed significantly between treatments in both soils. The δ13C values of CO2 were affected by relatively small CH4 fluxes in the fluctuating anaerobic/aerobic treatment. Effects of CH4 on δ13C values of CO2 were greater in the Oxisol due to its higher percent contribution of CH4 to total C mineralization(18%) than in the Mollisol (3%) during periods of elevated CH4 production. When CH4accounted for just 2% of total C mineralization, the δ13C values of CO2 differed from total C mineralization by 0.3–1‰, and by 1.4–4.8‰ when CH4 was 10% of C mineralization. These differences are highly significant when interpreting natural abundance δ13C data. Small CH4fluxes may strongly alter the δ13C values of CO2 relative to total mineralized C. A broad range of mineral and peatland soils can experience temporary oxygen deficits. In these dynamic redox environments, the δ13C values of CO2 should be interpreted with caution and ideally combined with δ13C of CH4 when partitioning sources and mechanisms of soil respiration.

Keywords
Carbon dioxide stable isotopes, Isotope mass balance, Methane isotopic fractionation, Redox fluctuation, Aerobic/anaerobic processes, Wetland

Comments
This is a manuscript of an article published as Huang, Wenjuan, and Steven J. Hall. "Large impacts of small methane fluxes on carbon isotope values of soil respiration." *Soil Biology and Biochemistry* 124 (2018): 126-133. doi: 10.1016/j.soilbio.2018.06.003. Posted with permission.

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

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ABSTRACT

Carbon dioxide isotope ($\delta^{13}C$ of CO_2) analysis is increasingly used to address a broad range of questions involving soil C dynamics and respiration sources. However, attaining $\delta^{13}C$ 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 $\delta^{13}C$ values of CO_2 due to large kinetic fractionations during CH_4 production. Thus, the $\delta^{13}C$ 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 $\delta^{13}C$ values of CO_2, we incubated a Mollisol and Oxisol amended with C_4-derived plant litter for 90 days under two headspace 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 $\delta^{13}C$ 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 $\delta^{13}C$ of CO_2 differed significantly between treatments in both soils. The $\delta^{13}C$ values of CO_2 were affected by relatively small CH_4 fluxes in the fluctuating anaerobic/aerobic treatment. Effects of CH_4 on $\delta^{13}C$ values of CO_2 were greater in the Oxisol due to its higher percent contribution of CH_4 to total C mineralization (18.22%) than in the Mollisol (3.25%) during periods of elevated CH_4 production. When CH_4 accounted for just 2% of total C mineralization, the $\delta^{13}C$ 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 $\delta^{13}C$ data. Small CH_4 fluxes may strongly alter the $\delta^{13}C$ 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 $\delta^{13}C$ values of CO_2 should be interpreted with caution and ideally combined with $\delta^{13}C$ of CH_4 when partitioning sources and mechanisms of soil respiration.

***Keywords*:** Carbon dioxide stable isotopes; Isotope mass balance; Methane isotopic fractionation; Redox fluctuation; Aerobic/anaerobic processes; Wetland

1. Introduction

Over the recent decades, stable carbon isotope ($\delta^{13}C$) 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., 1988; Ehleringer et al., 2000). For instance, measurements of $\delta^{13}C$ 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 $\delta^{13}C$ values of soil respiration is thus important for our understanding of soil and ecosystem C dynamics.

The $\delta^{13}C$ values of soil respiration are often thought to reflect $\delta^{13}C$ 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 $\delta^{13}C$ values of CO_2. When CO_2 co-occurs with methane (CH_4), the $\delta^{13}C$ 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: $\varepsilon = ((1000 + \delta^{13}C_C)/(1000 + \delta^{13}C_{CH_4}) - 1) \times 1000 \approx \delta^{13}C_C - \delta^{13}C_{CH_4}$ (Hayes, 1993), where $\delta^{13}C_C$ and $\delta^{13}C_{CH_4}$ are $\delta^{13}C$ values of the C source (either CO_2 or acetate) and CH_4, respectively. During methanogenesis, both the hydrogenotrophic (CO_2 reduction; $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$) and acetoclastic

(acetate fermentation; $CH_3COOH \rightarrow CH_4 + CO_2$) 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 $\delta^{13}C$ values in CH_4 relative to the C substrate (either CO_2 or acetate). By mass balance, residual CO_2 from 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 (ε = 3 – 30‰; Happell et al., 1994), resulting in higher $\delta^{13}C$ values of CH_4 and lower $\delta^{13}C$ values of CO_2 (Fig. 1). Hence, it is clearly important to consider CH_4 fractionation effects on $\delta^{13}C$ 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 $\delta^{13}C$ values of CO_2 than bulk soil $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ value of total mineralized C (CO_2 + CH_4) can be calculated as: $\delta^{13}C_{\mathrm{TC}} = P_{\mathrm{CH_4}}/100 \times \delta^{13}C_{\mathrm{CH_4}} + (1 - P_{\mathrm{CH_4}}/100) \times \delta^{13}C_{\mathrm{CO_2}}$, 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 $\delta^{13}C_{\mathrm{TC}} - \delta^{13}C_{\mathrm{CO_2}} =$

$-P_{CH_4}/100 \times (\delta^{13}C_{CO_2} - \delta^{13}C_{CH_4})$ to reflect the impact of CH_4 on the $\delta^{13}C$ value of CO_2 (ε_{TC-CO_2}). According to the above fractionation factors, the difference between $\delta^{13}C$ 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 ($\delta^{13}C_{CO_2} - \delta^{13}C_{CH_4}$) in soils with sporadic temporal or spatial O_2 limitation. Preliminary calculations suggest that small CH_4 fluxes could significantly impact the interpretation of $\delta^{13}C$ values of CO_2. If we assume a difference in $\delta^{13}C$ values of CH_4 and CO_2 of 30‰, a 5% contribution of CH_4 to total mineralized C would result in $\delta^{13}C$ 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 $\delta^{13}C$ budgets, where differences < 1‰ 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 $\delta^{13}C$ values when using $\delta^{13}C$ of CO_2 to understand C cycling processes. However, co-occurring measurements of $\delta^{13}C$ 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 $\delta^{13}C$ values of CO_2, CH_4 and total mineralized C (CO_2 + CH_4). We hypothesized that the fluctuating anaerobic/aerobic treatment would alter $\delta^{13}C$ values of CO_2 relative to $\delta^{13}C$ of soil mineralized C to a significant extent for ecological interpretation (i.e., one – several ‰) when relatively small CH_4 fluxes (P_{CH_4} ~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 volcaniclastic 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 $\delta^{13}C$ analysis method

The $\delta^{13}C$ 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 $\delta^{13}C$ dynamics over prolonged experiments (Krüger et al., 2002; Zhang et al., 2012). Alternatively, tunable diode laser absorption spectrometry (TDLAS) has been increasingly used for measuring $\delta^{13}C$ values of soil respiration (Marron et al., 2009; Bowling et al., 2015) due to its rapid measurement, low cost and relative analytical simplicity. This method has also been applied to direct measurements of $\delta^{13}C$ values of CH_4 (Bergamaschi et al., 1994). We recently developed a high-throughput method for measuring $\delta^{13}C$ values of CO_2 in small gas samples (Hall et al., 2017). Here, we applied a variation of this method to analyze $\delta^{13}C$ values of CH_4 by adding an in-line CO_2 trap and furnace to combust CH_4 to CO_2, which enabled $\delta^{13}C$ measurements of both gases on the same TDLAS instrument using separate replicate samples. This enabled relatively high measurement intensity (total n = 540) compared to previous incubation studies.

2.3. Initial soil chemical analysis

Soil pH (1:2.5 ratio of soil:deionized water) was 8.27 for the Mollisol and 5.03 for the Oxisol. The elevated pH value of the Mollisol indicated the presence of carbonate, which could potentially influence the $\delta^{13}C$ values of CO_2 following dissolution. We measured carbonate mass and its $\delta^{13}C$ values before incubation using a method modified from Amundson et al. (1988). In brief, air-dried and ground subsamples (~0.05 g) of the Mollisol were added to 100-ml bottles capped with Teflon septa sealed with aluminum crimps, and then flushed with ultra-zero (CO_2-

free) air for 15 min at 500 mL min^{-1}. Two mL of 3 M HCl was injected to each capped bottle with a gas-tight syringe. The bottles were shaken for 30 min. Five mL of gas collected from the headspace of each bottle was injected to a tunable diode laser absorption spectrometer (TDLAS; TGA200A; Campbell Scientific, Logan, UT, USA) to measure CO_2 concentration and its $\delta^{13}C$ value (Hall et al., 2017). The carbonate concentration in the Mollisol was 4.75 mg g^{-1}, and its $\delta^{13}C$ value was -2.08‰.

Bulk soil C and its $\delta^{13}C$ value were determined to be 38.9 mg g^{-1} and -19.6‰ in the Mollisol and 44.8 mg g^{-1} and -28.4‰ in the Oxisol, respectively, measured by an elemental analyzer interfaced with an isotope ratio mass spectrometer (ThermoFinnigan Delta Plus XL, Waltham, MA) at Iowa State University. For the Mollisol, soil organic C was 34.2 mg g^{-1} after accounting for the carbonate contribution to bulk soil C. A two-source mixing model was used to calculate $\delta^{13}C$ value of soil organic C ($\delta^{13}C_{SOC}$): $\delta^{13}C_{\mathrm{SOC}} = (C_{bulk} \times \delta^{13}C_{bulk} - C_{carb} \times \delta^{13}C_{\mathrm{carb}})/(C_{bulk} - C_{carb})$, where C_{bulk} and C_{carb} are the concentrations of bulk C and carbonate, respectively. Thus, the $\delta^{13}C$ value of SOC in the Mollisol was -22.0‰.

2.4. Laboratory incubation

Soils were gently homogenized after coarse roots and macrofauna were removed. Subsamples of fresh soils (5 g dry mass equivalent) were amended with 0.5 g finely ground leaf tissue of *Andropogon gerardii* (big bluestem, a C_4 grass with a $\delta^{13}C$ value of -13.3‰) that was harvested shortly after senescence. Litter was added to ameliorate short-term C limitation of microbial metabolism (Chacon et al., 2006). Soil samples

were mixed with litter and deionized water at field moisture capacity (0.46 g H_2O g^{-1} soil for the Mollisol and 1.01 g H_2O g^{-1} soil for the Oxisol), which was experimentally determined by saturating soils in the lab and measuring gravimetric water content following 48 hours of drainage. Each replicate soil sample was placed in an open 50 ml centrifuge tube and incubated in a glass jar (946 mL) sealed with a gas-tight aluminum lid equipped with butyl septa for headspace gas purging and sampling.

The soil samples received two headspace treatments: a fluctuating anaerobic/aerobic treatment with four days of N_2 alternating with four days of CO_2-free air, and a static aerobic treatment with CO_2-free air (control). Each treatment had three replicates for both the Mollisol and Oxisol. According to the above treatments, each jar was flushed with the appropriate gas for 15 min at 500 mL min^{-1} every two days immediately following each headspace gas measurement (described below). Purge gases (CO_2-free air or N_2) were humidified to minimize moisture lost during headspace flushing. Additional water was added as necessary by recording the mass of each sample during the incubation at eight-day intervals. The samples were incubated in the dark at 23 °C for 90 days.

2.5. Gas sample analysis

We collected gas samples for measurements of CO_2 and CH_4 concentrations and their $\delta^{13}C$ values immediately prior to headspace flushing, enabling us to quantify cumulative gaseous C losses and their $\delta^{13}C$ values over the entire 90-day experiment. A 5-mL sample was collected via a gas-tight syringe at two-day intervals and directly

injected into the TDLAS via an ultra-zero grade CO_2-free air carrier gas to measure the CO_2 concentration and its $\delta^{13}C$ value (Hall et al., 2017). Gas samples were collected from the fluctuating anaerobic/aerobic treatment at two-day intervals to measure CH_4 concentrations by gas chromatography (GC) with a flame ionization detector (GC-2014, Shimadzu, Columbia, MD) and the $\delta^{13}C$ of CH_4 by TDLAS, as described below. For the control, as the percent contribution of CH_4 to C mineralization measured in the static aerobic control averaged only 1.1%, gas samples were collected at four-day intervals to measure CH_4 concentrations by GC, and CH_4 production over two-day intervals was estimated as the average between consecutive four-day measurements. Here, we did not measure $\delta^{13}C$ values of CH_4 in the control due to its low contribution of CH_4 to C mineralization. Thus, we estimated $\delta^{13}C$ values of CH_4 in the control using the values measured during the aerobic phase of the fluctuating anaerobic/aerobic treatment when CH_4 percentage was equal to or lower than the maximum of the CH_4 percentage in the control.

2.6. $\delta^{13}C$ values of CH_4

We analyzed the $\delta^{13}C$ values of CH_4 by TDLAS following its oxidation to CO_2. The TDLAS sample inlet was connected to ultra-zero grade CO_2-free air used as a carrier gas. The gas sample was transported by the carrier gas through soda lime and magnesium perchlorate to remove CO_2 and water vapor. The sample then flowed into a ceramic tube in a combustion furnace. The ceramic tube was filled with oxidation catalyst (palladium powder and quartz wool) which quantitatively oxidized CH_4 to

CO_2 at ~800 °C (Fisher et al., 2005). The CO_2 was then introduced into the TDLAS to independently measure [^{12}C] and [^{13}C] mole fractions of CO_2 (Hall et al., 2017). The sample flow rate was 50 mL min^{-1}. Individual gas samples (5 mL) were injected by a polypropylene syringe with a Luer stopcock and a 25-gauge needle.

Three $\delta^{13}C$ standards for CH_4 (T-iso2, T-iso3 and L-iso1) purchased from Isometric Instruments (Victoria, British Columbia, Canada) were used for calibration. Two of the standards ([CH_4] = 250 μmol mol^{-1} and $\delta^{13}C$ value of CH_4 = -38.3 ± 0.2‰ for T-iso3; [CH_4] = 2500 μmol mol^{-1} and $\delta^{13}C$ value of CH_4 = -66.5 ± 0.2‰ for L-iso1) and ultra-zero air were used to produce calibration curves for [$^{12}CO_2$] and [$^{13}CO_2$] for calculation of $\delta^{13}C$ values during post-processing. Multiple samples diluted from the T-iso2 standard ([CH_4] = 2.5% and $\delta^{13}C$ value of CH_4 = -38.3 ± 0.2‰) by ultra-zero air were analyzed to independently characterize the accuracy and precision of our method. Samples from an additional NIST-traceable standard with [CH_4] = 100.9 μmol mol^{-1} were combusted over a temperature range of 765 – 840 °C to test the impact of the furnace temperature on measured $\delta^{13}C$ values of CH_4. To further assess method precision and accuracy, replicates of 32 gas samples were also analyzed for $\delta^{13}C$ values of CH_4 at the UC Davis Stable Isotope Facility using a ThermoScientific Precon concentration unit interfaced to a ThermoScientific Delta V Plus isotope ratio mass spectrometer (ThermoScientifc, Bremen, Germany).

2.7. Data processing

The peak heights measured after sample injection on the TDLAS were used for

subsequent calibration and calculation of $\delta^{13}C$ values as described in detail in Hall et al. (2017), as calculations based on peak areas yielded lower precision. Peak heights were calculated in reference to the baseline prior to each individual sample. Relationships between peak heights and known CH_4 mole fractions were linear across the range of standards used here (0 – 2500 μmol mol^{-1}). Separate linear regressions were used for [$^{12}CO_2$] and [$^{13}CO_2$] calibration. The $\delta^{13}C$ values of CO_2 and CH_4 were reported in ‰ following the convention: $\delta^{13}C = 1000 \times (R_{\mathrm{sample}}/RPDB - 1)$, where R is the molar ratio of $^{13}C/^{12}C$ and RPDB is $^{13}C/^{12}C$ of the Vienna Pee Dee Belemnite standard.

We calculated the stable C isotope separation between CO_2 and CH_4 for a given gas sample as follows: $\varepsilon_{\mathrm{CO_2-CH_4}} = \delta^{13}C_{\mathrm{CO_2}} - \delta^{13}C_{\mathrm{CH_4}}$. The difference between the $\delta^{13}C$ values of total mineralized C and CO_2 was defined here as $\varepsilon_{\mathrm{TC-CO_2}} = \delta^{13}C_{\mathrm{TC}} - \delta^{13}C_{\mathrm{CO_2}}$. Thus, $\varepsilon_{\mathrm{TC-CO_2}}$ can be interpreted as the impact of CH_4 on the $\delta^{13}C$ value of CO_2, which is simplified to $\varepsilon_{\mathrm{TC-CO_2}} = -P_{\mathrm{CH_4}}/100 \times \varepsilon_{\mathrm{CO_2-CH_4}}$. The cumulative $\delta^{13}C$ values of CO_2, weighted by their relative mass fluxes, were calculated as: $\delta^{13}C_{\mathrm{cumCO_2}} = \sum_{i=0}^{90}(\delta^{13}C_{\mathrm{CO_2}} \times F_{\mathrm{CO_2}})/\sum_{i=0}^{90} F_{\mathrm{CO_2}}$, where F_{CO_2} is the flux of CO_2 over each sampling interval *i*. The cumulative $\delta^{13}C$ value of total mineralized C was calculated as: $\delta^{13}C_{\mathrm{cumTC}} = \sum_{i=0}^{90}(\delta^{13}C_{\mathrm{CO_2}} \times F_{\mathrm{CO_2}} + \delta^{13}C_{\mathrm{CH_4}} \times F_{\mathrm{CH_4}})/\sum_{i=0}^{90}(F_{\mathrm{CO_2}} + F_{\mathrm{CH_4}})$, where F_{CH_4} is the flux of CH_4.

2.8. Statistical analysis

We tested the effects of the treatments and different phases of the fluctuating

anaerobic/aerobic treatment (anaerobic versus aerobic) on the $\delta^{13}C$ values of CH_4, CO_2 and total mineralized C, $\varepsilon_{CO_2-CH_4}$ and ε_{TC-CO_2} for each soil using a mixed-effects model, in which treatments/phases were treated as a fixed effect and samples as a random effect to account for repeated measurements. Statistically significant differences were accepted at $P < 0.05$. Mean values ± standard deviations were reported throughout the text. All statistical analyses were conducted with the R statistical package (R Core Team, 2018).

3. Results

3.1 Method performance for $\delta^{13}C$ value of CH_4 measurements by TDLAS

We first verified the accuracy and precision of the $\delta^{13}C$ value of CH_4 measurements. The $\delta^{13}C$ values of the CH_4 standard were not affected by combustion temperature at which CH_4 was oxidized to CO_2 between 765 and 840 °C. Subsequent gas samples thus were analyzed within this range of temperature. The $\delta^{13}C$ values of a subset of CH_4 samples with concentrations ranging from 38 to 5279 μmol mol^{-1} measured by TDLAS agreed well with those measured via isotope ratio mass spectrometry, with a slope of 0.97 and an R^2-value of 0.86 (n = 32). We independently analyzed a CH_4 standard (with a known value of -38.3‰) with [CH_4] diluted to 27, 55, and 150 μmol mol^{-1}, which measured -37.75 ± 2.73‰, -39.30 ± 1.39‰ and -39.14 ± 0.62‰ (n = 9 for each; one σ standard deviation), respectively. The $\delta^{13}C$ values of the same standard diluted to 11 μmol mol^{-1} were more variable, measuring -41.65 ± 5.23‰.

3.2. $\delta^{13}C$ values of CH_4 and CO_2

The Mollisol and Oxisol showed differing patterns in $\delta^{13}C$ values of CH_4 between the aerobic and anaerobic phases of the fluctuating treatment (Fig. 2a). Values of $\delta^{13}C$ of CH_4 were significantly more negative during the anaerobic phase (-43.97 ± 4.03‰) than the aerobic phase (-38.14 ± 5.69‰; $P < 0.01$) in the Mollisol. The $\delta^{13}C$ values of CH_4 in the Oxisol varied little between anaerobic and aerobic phases, averaging -49.16 ± 5.81‰ for the anaerobic phase and -48.06 ± 5.03‰ for the aerobic phase. The mean $\delta^{13}C$ values of CH_4 in the control were -38.14‰ for the Mollisol and -48.24‰ for the Oxisol.

The $\delta^{13}C$ values of CO_2 in the control were relatively stable over time (-10.13 ± 0.72‰ for the Mollisol and -14.90 ± 0.52‰ for the Oxisol), while $\delta^{13}C$ values of CO_2 varied with soil type and time in the fluctuating anaerobic/aerobic treatment (Fig. 2b). In the control, the Mollisol exhibited significantly higher $\delta^{13}C$ values of CO_2 than the Oxisol ($P < 0.01$). The $\delta^{13}C$ values of CO_2 in the fluctuating anaerobic/aerobic treatment were significantly more positive under the anaerobic phase (-9.05 ± 3.28‰ for the Mollisol and -8.81 ± 2.61‰ for the Oxisol) than the aerobic phase (-13.49 ± 1.18‰ for the Mollisol and -11.10 ± 2.53‰ for the Oxisol; $P < 0.01$ for both). The Mollisol showed a greater $\delta^{13}C$ value of CO_2 in the fluctuating anaerobic/aerobic treatment on the 4th day (1.91 ± 0.92‰). After that, the $\delta^{13}C$ values of CO_2 in the fluctuating anaerobic/aerobic treatment became similar to the control and gradually decreased over time ($P < 0.01$), with significantly lower values than the control after

44 days ($P < 0.05$). For the Oxisol, the $\delta^{13}C$ value of CO_2 in the fluctuating anaerobic/aerobic treatment increased after 24 days, and was significantly more positive than in the control ($P < 0.01$). Overall, at the end of the experiment, the fluctuating anaerobic/aerobic treatment exhibited a significantly lower cumulative $\delta^{13}C$ value of CO_2 in the Mollisol (-11.22 ± 0.43‰; $P < 0.05$) but a significantly higher value in the Oxisol (-10.83 ± 0.47‰; $P < 0.01$) relative to the controls (-9.85 ± 0.80‰ for the Mollisol and -14.89 ± 0.94‰ for the Oxisol).

The $\varepsilon_{CO_2-CH_4}$ values varied between the soils and the different phases of the fluctuating anaerobic/aerobic treatment (Fig. 2c). On average, the Mollisol showed significantly lower $\varepsilon_{CO_2-CH_4}$ (29.79‰) than the Oxisol (38.65‰; $P < 0.01$). The anaerobic phase significantly increased $\varepsilon_{CO_2-CH_4}$ relative to the aerobic phase ($P < 0.01$), especially in the Mollisol (Fig. 2c). The mean $\varepsilon_{CO_2-CH_4}$ value in the anaerobic phase was 34.92 ± 5.64‰ for the Mollisol and 40.35 ± 5.17‰ for the Oxisol, and the aerobic phase was 24.65 ± 6.22‰ for the Mollisol and 36.96 ± 5.38‰ for the Oxisol.

3.3. $\delta^{13}C$ values of total mineralized C

The pattern of $\delta^{13}C$ values of total mineralized C was similar to $\delta^{13}C$ of CO_2 in the control, but not in the fluctuating anaerobic/aerobic treatment—especially for the Oxisol. The $\delta^{13}C$ values of total mineralized C in the control did not vary with time, and were significantly higher in the Mollisol (-10.60 ± 0.87‰) than in the Oxisol (-15.06 ± 0.95‰; $P < 0.01$). However, in the fluctuating anaerobic/aerobic treatment, the $\delta^{13}C$ values of total mineralized C in the Mollisol became much more negative

relative to $\delta^{13}C$ of CO_2 between 12 and 44 days, resulting in the $\delta^{13}C$ values of total mineralized C fluctuating between -14.56‰ and -9.22‰ after 12 days. After 24 days, the $\delta^{13}C$ of total mineralized C in the Oxisol became lower in the fluctuating anaerobic/aerobic treatment than in the control, and fluctuated between -20.03‰ and -13.83‰ (Fig. 3a). After 90 days, the cumulative $\delta^{13}C$ value of total mineralized C in the Mollisol was significantly lower in the fluctuating anaerobic/aerobic treatment (-11.65 ± 0.30‰) than in the control (-10.36 ± 0.45‰; $P < 0.05$). In contrast to the cumulative $\delta^{13}C$ value of CO_2, the cumulative $\delta^{13}C$ value of total mineralized C in the Oxisol did not significantly differ between treatments (-15.67 ± 0.28‰ for the fluctuating anaerobic/aerobic treatment and -15.05 ± 0.72‰ for the control).

Values of ε_{TC-CO_2}, which reflected the impact of CH_4 on the $\delta^{13}C$ values of CO_2, strongly varied with time in the fluctuating anaerobic/aerobic treatment but not in the control (Fig. 3b). For the control, the change in $\delta^{13}C$ value of CO_2 relative to total mineralized C was small in both soils (ε_{TC-CO_2} = -0.48 ± 0.30‰ for the Mollisol and -0.16 ± 0.25‰ for the Oxisol) due to a low cumulative percentage of CH_4 to total C mineralization (1.84% for the Mollisol and 0.48% for the Oxisol). However, the $\delta^{13}C$ of total mineralized C strongly differed from that of CO_2 in the fluctuating anaerobic/aerobic treatment ($P < 0.01$), especially in the Oxisol. In the Mollisol, ε_{TC-CO_2} was more negative under the anaerobic phase (ε_{TC-CO_2} = -1.04 ± 1.25‰) relative to the aerobic phase (ε_{TC-CO_2} = -0.14 ± 0.17‰) ($P < 0.01$). In the Oxisol, ε_{TC-CO_2} became more negative after 24 days ($P < 0.01$), ranging from -12.00‰ under the anaerobic phase to -3.88‰ under the aerobic phase.

Variations in ε_{TC-CO_2} were closely related to the percent contribution of CH_4 to C mineralization (defined here as CH_4 percentage; Fig. 3c). The CH_4 percentage in the control was always < 3.50% and <2.40% for the Mollisol and Oxisol, respectively, averaging 1.67% and 0.47%. The fluctuating anaerobic/aerobic treatment caused fluctuations in CH_4 percentage over time (Fig. 3c), especially in the Oxisol, due to relative changes in CO_2 and CH_4 production as a function of O_2 availability (Fig. 4). In the Mollisol, the fluctuating anaerobic/aerobic treatment produced higher CH_4 percentages between 12 and 44 days, ranging from 0.16% to 10.45% (mean of 3.25%), with significantly higher values during the anaerobic than the aerobic phases ($P < 0.01$). The fluctuating anaerobic/aerobic treatment in the Oxisol showed significantly greater CH_4 percentages than the control after 24 days ($P < 0.01$), ranging from 6.45% to 29.00% (mean of 18.22%).

To generalize our findings to other terrestrial mineral soils, we calculated the impacts of CH_4 percentage on ε_{TC-CO_2} across a range of CH_4 production and $\varepsilon_{CO_2-CH_4}$ values corresponding with our data (Table 1). We showed three scenarios corresponding with minimum, mean, and maximum observed $\varepsilon_{CO_2-CH_4}$ values and contributions of CH_4 to total C mineralization from 2% to 50%. If CH_4 production was < 2% of total C mineralization, we would expect ε_{TC-CO_2} of -0.96 – -0.28‰ based on an $\varepsilon_{CO_2-CH_4}$ between 14‰ and 48‰, respectively. The absolute magnitude of ε_{TC-CO_2} values increased sharply as the percent of CH_4 increased: -2.40 – -0.70‰ given CH_4 production of 5%, and so on (Table 1).

4. Discussion

We found that terrestrial soils exposed to brief periods (4 days) of anaerobic conditions can produce small but significant CH_4 fluxes which continue to a lesser extent during subsequent periods of O_2 exposure. Consistent with our hypothesis, even the relatively small CH_4 fluxes observed here significantly altered the $\delta^{13}C$ values of CO_2 (by as much as 12‰) relative to $\delta^{13}C$ of total soil C mineralization, and thus critically altered their ecological interpretation. This is crucially important in the context of studies which aim to decipher the source of respiration using small (i.e., 1 – 4‰) differences in $\delta^{13}C$ values associated with specific organic compounds (e.g., carbohydrates vs. lipids or lignin) or specific ecological sources (e.g., roots vs. microbes; Bowling et al., 2008). Many previous studies reporting measurements of $\delta^{13}C$ of soil respiration have been conducted in periodically wet, C-rich soils from terrestrial or ephemeral wetland ecosystems where small net CH_4 fluxes may have been present (i.e., conditions similar to our study). These include studies from boreal peatlands and arctic tundra where relatively small differences in $\delta^{13}C$ of CO_2 (several ‰) were interpreted in the context of identifying C substrates for CO_2 production (Dioumaeva et al., 2002; Oelbermann et al., 2008) and quantifying $\delta^{13}C$ values of ecosystem respiration (Natali et al., 2011; Hicks Pries et al., 2013), but where CH_4 was not apparently measured. Our study suggests the possibility that some of this reported isotopic variation, and the otherwise intriguing differences between $\delta^{13}C$ values of CO_2 and bulk soil $\delta^{13}C$, might derive from CH_4 production. In ecosystems where even small net positive CH_4 fluxes to the atmosphere occur (i.e.,

accounting for ~5% of total C mineralization), explicit consideration of CH_4 contributions to $\delta^{13}C$ mass balance appears necessary for robust interpretation of $\delta^{13}C$ of soil respiration.

4.1. Effects of dynamic redox environments on $\delta^{13}C$ values of CH_4 and CO_2

Few studies have conducted frequent measurements of $\delta^{13}C$ values of CO_2 during prolonged soil incubations (Breecker et al., 2015; Huang and Hall, 2017), and even fewer have measured $\delta^{13}C$ values of both CO_2 and CH_4 (Conrad and Claus, 2009). The relatively higher $\delta^{13}C$ values of CO_2 and CH_4 in the Mollisol than the Oxisol likely reflected their initial $\delta^{13}C$ values of SOC (-22.0‰ for the Mollisol, a mixed C_3-C_4 agroecosystem, and -28.4‰ for the Oxisol, a C_3 forest).

However, the temporal trends in the $\delta^{13}C$ values of CO_2 and CH_4 clearly reflected CH_4-driven C isotope fractionation under the fluctuating anaerobic/aerobic treatment. Under aerobic conditions, heterotrophic microbial respiration does not normally fractionate $\delta^{13}C$ values to a major extent (Ehleringer et al., 2000; Breecker et al., 2015). Accordingly, CO_2 produced in the control likely reflected the original $\delta^{13}C$ signature of soil organic C compounds from which CO_2 was derived. The relatively positive $\delta^{13}C$ value of CO_2 (-10.13‰) in the Mollisol relative to C_4 litter (-13.3‰) and soil organic C (-22.0‰) may be attributed to a small proportion of CO_2 generated by the dissolution of carbonate (-2.08‰). In comparison, the more positive $\delta^{13}C$ values of CO_2 under the anaerobic phase (-9.05‰ for the Mollisol and -8.81‰ for the Oxisol) coincided with higher CH_4 percentages, as is commonly observed in

consistently saturated wetland systems with much greater CH_4 production (Corbett et al., 2013; Berger et al., 2018). Compared with the relatively stable $\delta^{13}C$ value of CO_2 in the control, the mean $\delta^{13}C$ values of CH_4 under the anaerobic phase (-43.97‰ for the Mollisol and -49.16‰ for the Oxisol) were consistent with a mixture of hydrogenotrophic and acetoclastic methanogenesis (Blaser and Conrad, 2016). When CH_4 was produced under the anaerobic phase, the residual CO_2 was enriched in ^{13}C in order to balance the depleted ^{13}C of CH_4 (Fig. 1), leading to higher $\varepsilon_{CO_2-CH_4}$. On the other hand, CH_4 oxidation under the aerobic phase subsequently increased $\delta^{13}C$ values of CH_4 and thus decreased $\varepsilon_{CO_2-CH_4}$ (Fig. 1). Relative to the Oxisol, the larger variations in $\delta^{13}C$ values of CH_4 and $\varepsilon_{CO_2-CH_4}$ between the anaerobic and aerobic phases in the Mollisol suggested that CH_4 oxidation may have been more important. The mean difference in $\delta^{13}C$ values of CH_4 between the end of aerobic and anaerobic phases in the Mollisol (~6‰) was consistent with fractionation from CH_4 oxidation, within the range of typical values (< 10‰; Whiticar, 1999). Thus, fluctuations in the $\delta^{13}C$ values of CO_2 and CH_4 and $\varepsilon_{CO_2-CH_4}$ were determined by the combination of the contribution of methanogenesis to total C mineralization and CH_4 oxidation, as well as any potential shifts in C sources accompanying dynamic redox conditions (Huang and Hall, 2017).

In addition, dissolution of a small fraction of the isotopically heavy carbonate in the Mollisol likely contributed to a one-time pulse of CO_2 with very high $\delta^{13}C$ values at day 4 accompanying decreased pH under the anaerobic phase. Temporary decreases in pH in alkaline soils are commonly observed following the onset of anaerobic

conditions due to the transient accumulation of organic acids (Kirk, 2004).

Finally, the $\delta^{13}C$ values of CH_4 measured by our TDLAS method agreed well with conventional isotope ratio mass spectrometry (IRMS). The precision for continuous-flow IRMS can be < 0.2‰ (Yarnes, 2013), which is slightly better than our method. However, the TDLAS method provided an alternative to IRMS for $\delta^{13}C$ value of CH_4 measurement with high throughout (20 samples h^{-1}) at much lower operating costs, which facilitated the high-frequency use of C isotopes to understand the highly dynamic C mineralization in soils with O_2 fluctuations.

4.2. Impacts of CH_4 flux on $\delta^{13}C$ values of soil respiration

Total mineralized C had lower $\delta^{13}C$ values than CO_2 under fluctuating anaerobic/aerobic conditions, suggesting that the $\delta^{13}C$ values of CO_2 did not directly reflect the sources of mineralized C in soils that produced CH_4. Similarly, previous work showed that $\delta^{13}C$ values of CO_2 became more positive with increasing CH_4/CO_2 ratios (Hodgkins et al., 2014). A previous study (Holmes et al., 2015) showed that after incubating peat under anaerobic conditions for 48 days, CH_4 production representing 2% and 9% of total mineralized C resulted in ε_{TC-CO_2} values of -3.2‰ and -8.3‰, respectively, due to the dominance of hydrogenotrophic methanogenesis with very high enrichment factors (> 80‰). In the mineral soils examined here, the $\varepsilon_{CO_2-CH_4}$ values were smaller, suggesting that the influence of CH_4 fractionation on $\delta^{13}C$ values of CO_2 could potentially be neglected if CH_4 accounts for < 2%. This is consistent with our previous study examining CO_2 and CH_4 dynamics from another

Mollisol (Huang and Hall, 2017). In contrast, $\delta^{13}C$ values of soil respiration were strongly affected (ε_{TC-CO_2} up to -12‰) by the anaerobic/aerobic phase with CH_4 > 5% of total mineralized C, which supported our hypothesis. Thus, both the CH_4 percentages and $\varepsilon_{CO_2-CH_4}$ values are critical controls on $\delta^{13}C$ values of soil respiration.

4.3. Implications for ecosystem $\delta^{13}C$ dynamics

Net CH_4 production is traditionally thought to occur only after prolonged anaerobic conditions (Conrad, 1996), but our results showed that CH_4 production was highly significant even under short-duration O_2 fluctuations. This is consistent with previous reports of sporadic net CH_4 production from bulk aerobic terrestrial soils (Silver et al., 1999; von Fischer and Hedin, 2007; Liptzin et al., 2011; Hall et al., 2013). Similar to our study, net CH_4 production was also observed for several days after saturated soils became fully aerated (Ebrahimi and Or, 2017). Thus, CH_4 fractionation effects on $\delta^{13}C$ values of soil respiration should not necessarily be ignored even in bulk aerobic soils. For example, Hicks Pries (2013) found that the $\delta^{13}C$ value of respired CO_2 increased from ~ -26‰ to ~ -20‰ with depth (0 – 80 cm) in peatland soils incubated under aerobic conditions. In that study, the 6‰ increase in $\delta^{13}C$ value of CO_2 was interpreted as a shift in C sources from plant to microbial-derived C with depth, even as bulk soil $\delta^{13}C$ values varied little with depth (Hicks Pries et al., 2012). Our data suggest that a small increase in net CH_4 production with depth could also explain the observed increase in $\delta^{13}C$ value of CO_2 and the 4.8‰ difference between $\delta^{13}C$ values of CO_2 and bulk soil C in that study.

5. Conclusions

The results from this incubation experiment emphasized the necessity to have frequent measurements of CO_2 and CH_4 production and their $\delta^{13}C$ values in soils that experienced O_2 fluctuations, in order to better reflect the $\delta^{13}C$ values of total mineralized C. We found that short-duration fluctuations in O_2 availability produced small but significant CH_4 fluxes in the two mineral soils examined here, which strongly altered the $\delta^{13}C$ values of CO_2. We observed smaller differences in $\delta^{13}C$ values between CO_2 and CH_4 (14 – 48‰) than in previous studies of consistently saturated wetland soils. However, as hypothesized, when CH_4 represented > 5% of total soil C mineralization, consideration of $\delta^{13}C$ values of CH_4 was necessary for robust interpretation of $\delta^{13}C$ values of soil respiration in these dynamic redox environments.

Acknowledgements

We thank Anthony Mirabito, Lucio Reyes, Jessica Riojas, Ethan Rose, and Chenglong Ye for lab assistance, and Suzanne Ankerstjerne for assistance with IRMS analyses at Iowa State. Funding was provided by NSF DEB-1457805 and Iowa State University.

Competing interests: The authors declare no competing financial interests.

References

Amundson, R.G., Trask, J., Pendall, E., 1988. A rapid method of soil carbonate

analysis using gas chromatography. Soil Science Society of America Journal 52, 880–883. doi:10.2136/sssaj1988.03615995005200030050x.

Bergamaschi, P., Schupp, M., Harris, G.W., 1994. High-precision direct measurements of $^{13}CH_4/^{12}CH_4$ and $^{12}CH_3D/^{12}CH_4$ ratios in atmospheric methane sources by means of a long-path tunable diode laser absorption spectrometer. Applied Optics 33, 7704–7716. doi:10.1364/AO.33.007704

Berger, S., Praetzel, L.S.E., Goebel, M., Blodau, C., Knorr, K.-H., 2018. Differential response of carbon cycling to long-term nutrient input and altered hydrological conditions in a continental Canadian peatland. Biogeosciences 15, 885–903. doi:10.5194/bg-15-885-2018

Blaser, M., Conrad, R., 2016. Stable carbon isotope fractionation as tracer of carbon cycling in anoxic soil ecosystems. Current Opinion in Biotechnology, 41, 122–129. https://doi.org/10.1016/j.copbio.2016.07.001

Bowling D.R., Ballantyne A.P., Miller J.B., Burns S.P., Conway T.J., Menzer O., Stephens B.B., Vaughn B.H., 2014. Ecological processes dominate the ^{13}C land disequilibrium in a Rocky Mountain subalpine forest. Global Biogeochemical Cycles 28, 352–370. doi:10.1002/2013GB004686

Bowling, D.R., Egan, J.E., Hall, S.J., Risk, D.A., 2015. Environmental forcing does not induce diel or synoptic variation in the carbon isotope content of forest soil respiration. Biogeosciences 12, 5143–5160. doi:10.5194/bg-12-5143-2015

Bowling, D.R., Pataki, D.E., Randerson, J.T., 2008. Carbon isotopes in terrestrial ecosystem pools and CO_2 fluxes. New Phytologist 178, 24–40.

doi:10.1111/j.1469-8137.2007.02342.x

Breecker, D.O., Bergel, S., Nadel, M., Tremblay, M.M., Osuna-Orozco, R., Larson, T.E., Sharp, Z.D., 2015. Minor stable carbon isotope fractionation between respired carbon dioxide and bulk soil organic matter during laboratory incubation of topsoil. Biogeochemistry 123, 83–98. doi:10.1007/s10533-014-0054-3

Buss, H.L., Chapela Lara, M., Moore, O.W., Kurtz, A.C., Schulz, M.S., White, A.F., 2017. Lithological influences on contemporary and long-term regolith weathering at the Luquillo Critical Zone Observatory. Geochimica et Cosmochimica Acta 196, 224–251. https://doi.org/10.1016/j.gca.2016.09.038

Chacon, N., Silver, W.L., Dubinsky, E.A., Cusack, D.F., 2006. Iron reduction and soil phosphorus solubilization in humid tropical forests soils: The roles of labile carbon pools and an electron shuttle compound. Biogeochemistry 78, 67–84. doi:10.1007/s10533-005-2343-3

Collins, H.P., Elliott, E.T., Paustian, K., Bundy, L.G., Dick, W.A., Huggins, D.R., Smucker, A.J.M., Paul, E.A., 2000. Soil carbon pools and fluxes in long-term corn belt agroecosystems. Soil Biology and Biochemistry 32, 157–168. doi:10.1016/S0038-0717(99)00136-4

Conrad, R., 1996. Soil microorganisms as controllers of atmospheric trace gases (H_2, CO, CH_4, OCS, N_2O, and NO). Microbiological Reviews 60, 609–640.

Conrad, R., Claus, P., 2009. Characterization of stable isotope fractionation during methane production in the sediment of a eutrophic lake, Lake Dagow,

Germany. Limnology and Oceanography 54, 457–471. doi:10.4319/lo.2009.54.2.0457

Corbett, J.E., Tfaily, M.M., Burdige, D.J., Cooper, W.T., Glaser, P.H., Chanton, J.P., 2013. Partitioning pathways of CO_2 production in peatlands with stable carbon isotopes. Biogeochemistry 114, 327–340. doi:10.1007/s10533-012-9813-1

Dioumaeva, I., Trumbore, S., Schuur, E.A.G., Goulden, M.L., Litvak, M., Hirsch, A.I., 2002. Decomposition of peat from upland boreal forest: Temperature dependence and sources of respired carbon. Journal of Geophysical Research: Atmospheres 107, 8222. doi:10.1029/2001JD000848

Ebrahimi, A., Or, D., 2017. Dynamics of soil biogeochemical gas emissions shaped by remolded aggregate sizes and carbon configurations under hydration cycles. Global Change Biology 24, e378–e392. doi:10.1111/gcb.13938

Ehleringer, J.R., Buchmann, N., Flanagan, L.B., 2000. Carbon isotope ratios in belowground carbon cycle processes. Ecological Applications 10, 412–422. https://doi.org/10.1890/1051-0761(2000)010[0412:CIRIBC]2.0.CO;2

Fisher R., Lowry D., Wilkin O., Sriskantharajah S., Nisbet E.G., 2005. High-precision, automated stable isotope analysis of atmospheric methane and carbon dioxide using continuous-flow isotope-ratio mass spectrometry. Rapid Communications in Mass Spectrometry 20, 200–208. doi:10.1002/rcm.2300

Kirk G., 2004. The biogeochemistry of submerged soils. John Wiley & Sons Ltd, Chichester, UK.

Hall, S.J., McDowell, W.H., Silver, W.L., 2013. When wet gets wetter: Decoupling of

moisture, redox biogeochemistry, and greenhouse gas fluxes in a humid tropical forest soil. Ecosystems 16, 576–589. doi:10.1007/s10021-012-9631-2

Hall, S.J., Treffkorn, J., Silver, W.L., 2014. Breaking the enzymatic latch: impacts of reducing conditions on hydrolytic enzyme activity in tropical forest soils. Ecology 95, 2964–2973. doi:https://doi.org/10.1890/13-2151.1

Hall, S.J., Baker, M.A., Jones, S.B., Stark, J.M., Bowling, D.R., 2016. Contrasting soil nitrogen dynamics across a montane meadow and urban lawn in a semi-arid watershed. Urban Ecosystems 19, 1083–1101. doi:10.1007/s11252-016-0538-0

Hall S.J., Huang W., Hammel K.E., 2017. An optical method for carbon dioxide isotopes and mole fractions in small gas samples: Tracing microbial respiration from soil, litter, and lignin. Rapid Communications in Mass Spectrometry 31, 1938–1946. doi:10.1002/rcm.7973

Hanson, P.J., Edwards, N.T., Garten, C.T., Andrews, J.A., 2000. Separating root and soil microbial contributions to soil respiration: A review of methods and observations. Biogeochemistry 48, 115–146. doi:10.1023/A:1006244819642

Happell, J.D., Chanton, J.P., Showers, W.S., 1994. The influence of methane oxidation on the stable isotopic composition of methane emitted from Florida swamp forests. Geochimica et Cosmochimica Acta 58, 4377–4388. doi:10.1016/0016-7037(94)90341-7

Hayes, J.M., 1993. Factors controlling ^{13}C contents of sedimentary organic compounds: Principles and evidence. Marine Geology, Marine Sediments,

Burial, Pore Water Chemistry, Microbiology and Diagenesis 113, 111–125. doi:10.1016/0025-3227(93)90153-M

Hicks Pries, C.E., Schuur, E.A.G., Crummer, K.G., 2013. Thawing permafrost increases old soil and autotrophic respiration in tundra: Partitioning ecosystem respiration using $\delta^{13}C$ and $\Delta^{14}C$. Global Change Biology 19, 649–661. doi:10.1111/gcb.12058

Hicks Pries, C.E., Schuur, E.A.G., Natali, S.M., Crummer, K.G., 2015. Old soil carbon losses increase with ecosystem respiration in experimentally thawed tundra. Nature Climate Change 6, 214–218. doi:10.1038/nclimate2830

Hodgkins, S.B., Tfaily, M.M., McCalley, C.K., Logan, T.A., Crill, P.M., Saleska, S.R., Rich, V.I., Chanton, J.P., 2014. Changes in peat chemistry associated with permafrost thaw increase greenhouse gas production. Proceedings of the National Academy of Sciences 111, 5819–5824. doi:10.1073/pnas.1314641111

Holmes, M.E., Chanton, J.P., Tfaily, M.M., Ogram, A., 2015. CO_2 and CH_4 isotope compositions and production pathways in a tropical peatland. Global Biogeochemical Cycles 29, 1–18. doi:10.1002/2014GB004951

Hornibrook, E.R.C., Longstaffe, F.J., Fyfe, W.S., 2000. Evolution of stable carbon isotope compositions for methane and carbon dioxide in freshwater wetlands and other anaerobic environments. Geochimica et Cosmochimica Acta 64, 1013–1027. doi:https://doi.org/10.1016/S0016-7037(99)00321-X

Huang, W., Hall, S.J., 2017. Elevated moisture stimulates carbon loss from mineral

soils by releasing protected organic matter. Nature Communications 8, 1774. doi:10.1038/s41467-017-01998-z

Jarecke, K.M., Loecke, T.D., Burgin, A.J., 2016. Coupled soil oxygen and greenhouse gas dynamics under variable hydrology. Soil Biology and Biochemistry 95, 164–172. doi:10.1016/j.soilbio.2015.12.018

Krüger, M., Eller, G., Conrad, R., Frenzel, P., 2002. Seasonal variation in pathways of CH_4 production and in CH_4 oxidation in rice fields determined by stable carbon isotopes and specific inhibitors. Global Change Biology 8, 265–280. doi:10.1046/j.1365-2486.2002.00476.x

Liptzin, D., Silver, W.L., Detto, M., 2011. Temporal dynamics in soil oxygen and greenhouse gases in two humid tropical forests. Ecosystems 14, 171–182. doi:10.1007/s10021-010-9402-x

Logsdon, S.D., 2015. Event- and site-specific soil wetting and seasonal change in amount of soil water. Soil Science Society of America Journal 79, 730–741. doi:10.2136/sssaj2014.08.0327

Marron, N., Plain, C., Longdoz, B., Epron, D., 2009. Seasonal and daily time course of the ^{13}C composition in soil CO_2 efflux recorded with a tunable diode laser spectrophotometer (TDLS). Plant and Soil 318, 137–151. doi:10.1007/s11104-008-9824-9

Moyes, A.B., Gaines, S.J., Siegwolf, R.T.W., Bowling, D.R., 2010. Diffusive fractionation complicates isotopic partitioning of autotrophic and heterotrophic sources of soil respiration. Plant, Cell & Environment 33, 1804–

1819. doi:10.1111/j.1365-3040.2010.02185.x

Moyes, A.B., Bowling, D.R., 2013. Interannual variation in seasonal drivers of soil respiration in a semi-arid Rocky Mountain meadow. Biogeochemistry 113, 683–697. doi:10.1007/s10533-012-9797-x

Natali, S.M., Schuur, E.A.G., Trucco, C., Hicks Pries, C.E., Crummer, K.G., Baron Lopez, A.F., 2011. Effects of experimental warming of air, soil and permafrost on carbon balance in Alaskan tundra. Global Change Biology 17, 1394–1407. doi:10.1111/j.1365-2486.2010.02303.x

O'Connell, C.S., Ruan, L., Silver, W.L., 2018. Drought drives rapid shifts in tropical rainforest soil biogeochemistry and greenhouse gas emissions. Nature Communications 9, 1348. doi:10.1038/s41467-018-03352-3

Oelbermann, M., English, M., Schiff, S.L., 2008. Evaluating carbon dynamics and microbial activity in arctic soils under warmer temperatures. Canadian Journal of Soil Science 88, 31–44. doi:10.4141/CJSS07060

Penning Holger, Plugge Caroline M., Galand Pierre E., Conrad Ralf, 2005. Variation of carbon isotope fractionation in hydrogenotrophic methanogenic microbial cultures and environmental samples at different energy status. Global Change Biology 11, 2103–2113. doi:10.1111/j.1365-2486.2005.01076.x

Pries, C.E.H., Schuur, E.A.G., Crummer, K.G., 2012. Holocene carbon stocks and carbon accumulation rates altered in soils undergoing permafrost thaw. Ecosystems 15, 162–173. doi:10.1007/s10021-011-9500-4

R Core Team, 2018. R: A language and environment for statistical computing. R

Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org

Sexstone, A.J., Revsbech, N.P., Parkin, T.B., Tiedje, J.M., 1985. Direct measurement of oxygen profiles and denitrification rates in soil aggregates 1. Soil Science Society of America Journal 49, 645–651. doi:10.2136/sssaj1985.03615995004900030024x

Silver, W.L., Lugo, A.E., Keller, M., 1999. Soil oxygen availability and biogeochemistry along rainfall and topographic gradients in upland wet tropical forest soils. Biogeochemistry 44, 301–328. doi:10.1023/A:1006034126698

Tu, K., Dawson, T., 2005. Partitioning ecosystem respiration using stable carbon isotope analyses of CO_2. In: Flanagan, L., Ehleringer, J., Pataki, D. (Eds.), Stable Isotopes and Biosphere-Atmosphere Interactions: Processes and Biological Controls. Elsevier, San Diego, pp. 125–153.

Vestergård, M., Reinsch, S., Bengtson, P., Ambus, P., Christensen, S., 2016. Enhanced priming of old, not new soil carbon at elevated atmospheric CO_2. Soil Biology and Biochemistry 100, 140–148. doi:10.1016/j.soilbio.2016.06.010

von Fischer J.C., Hedin L.O., 2007. Controls on soil methane fluxes: Tests of biophysical mechanisms using stable isotope tracers. Global Biogeochemical Cycles 21, GB2007. doi:10.1029/2006GB002687

Whiticar, M.J., 1999. Carbon and hydrogen isotope systematics of bacterial formation

and oxidation of methane. Chemical Geology 161, 291–314. doi:10.1016/s0009-2541(99)00092-3

Yang, W.H., Silver, W.L., 2016. Net soil-atmosphere fluxes mask patterns in gross production and consumption of nitrous oxide and methane in a managed ecosystem. Biogeosciences 13, 1705–1715. https://doi.org/10.5194/bg-13-1705-2016

Yarnes, C., 2013. $\delta^{13}C$ and $\delta^{2}H$ measurement of methane from ecological and geological sources by gas chromatography/combustion/pyrolysis isotope-ratio mass spectrometry. Rapid Communications in Mass Spectrometry 27, 1036–1044. doi:10.1002/rcm.6549

Zhang, G., Ji, Y., Ma, J., Xu, H., Cai, Z., Yagi, K., 2012. Intermittent irrigation changes production, oxidation, and emission of CH_4 in paddy fields determined with stable carbon isotope technique. Soil Biology and Biochemistry 52, 108–116. doi:https://doi.org/10.1016/j.soilbio.2012.04.017

Table 1 Differences in $\delta^{13}C$ values between total mineralized C and CO_2 (ε_{TC-CO_2}) as a function of the percentage of CH_4 to total mineralized C (P_{CH_4}). $\varepsilon_{TC-CO_2} = -P_{CH_4}/100 \times \varepsilon_{CO_2-CH_4}$, where $\varepsilon_{CO_2-CH_4}$ is C isotope separation between CO_2 and CH_4. The values of ε_{min}, ε_{mean} and ε_{max} were representative of our data. ε_{min}, minimum of $\varepsilon_{CO_2-CH_4}$; ε_{mean}, mean of $\varepsilon_{CO_2-CH_4}$; ε_{max}, maximum of $\varepsilon_{CO_2-CH_4}$.

CH_4 percentage (%)	ε_{min} (‰) 14	ε_{mean} (‰) 32	ε_{max} (‰) 48
2	-0.28	-0.64	-0.96
5	-0.7	-1.6	-2.4
10	-1.4	-3.2	-4.8
30	-4.2	-9.6	-14.4
50	-7	-16	-24

Figure captions

Fig. 1 Schematic of processes affecting C isotope ratios ($\delta^{13}C$ values) of CH_4 and CO_2 in fluctuating anaerobic/aerobic soils. The numbers on the lines indicate C isotope enrichment 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 $\delta^{13}C$ values of CO_2; and the line in blue indicates that fractionation decreased $\delta^{13}C$ values of CO_2.

Fig. 2 Carbon isotopes ($\delta^{13}C$ values) of CH_4 (a), CO_2 (b), and their difference ($\varepsilon_{CO_2-CH_4}$) (c) in the Mollisol and Oxisol under a fluctuating anaerobic/aerobic treatment and a static aerobic condition (control). $\varepsilon_{CO_2-CH_4} = \delta^{13}C_{CO_2} - \delta^{13}C_{CH_4}$. The solid line indicates aerobic conditions, and the dashed line indicates anaerobic conditions. The error bars indicate SD (n = 3 for each treatment).

Fig. 3 Carbon isotope ($\delta^{13}C$ values) of total mineralized C (CH_4 and CO_2) (a), the difference in $\delta^{13}C$ values between total mineralized C and CO_2 (ε_{TC-CO_2}) (b), and CH_4 percentage contribution to total mineralized C (c) in the Mollisol and Oxisol under a fluctuating anaerobic/aerobic treatment and a static aerobic condition (control). The solid line indicates aerobic conditions, and the dashed line indicates anaerobic

conditions. The error bars indicate SD (n = 3 for each treatment).

Fig. 4 Production rate of CH_4 and CO_2 in the Mollisol and Oxisol under a fluctuating anaerobic/aerobic treatment and a static aerobic condition (control). The solid line indicates aerobic conditions, and the dashed line indicates anaerobic conditions. The error bars indicate SD (n = 3 for each treatment).

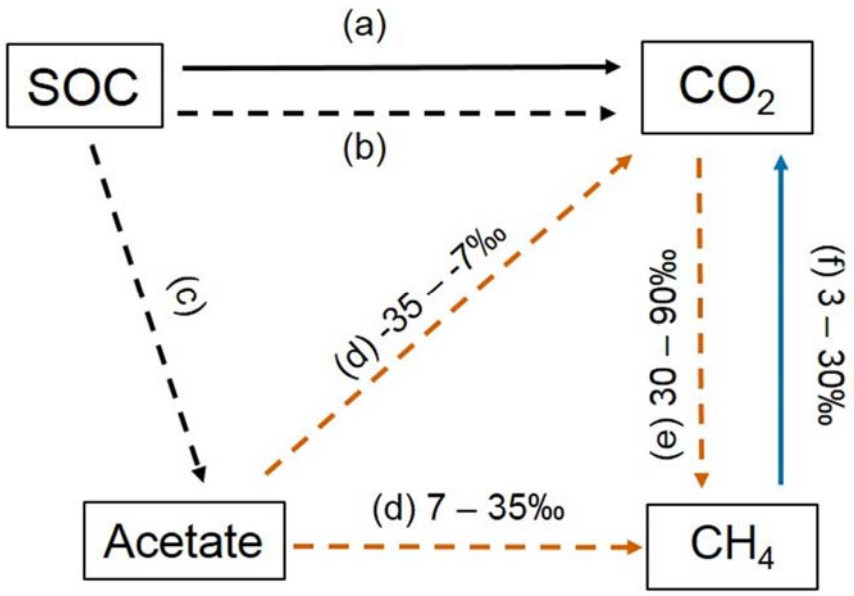

Fig. 1

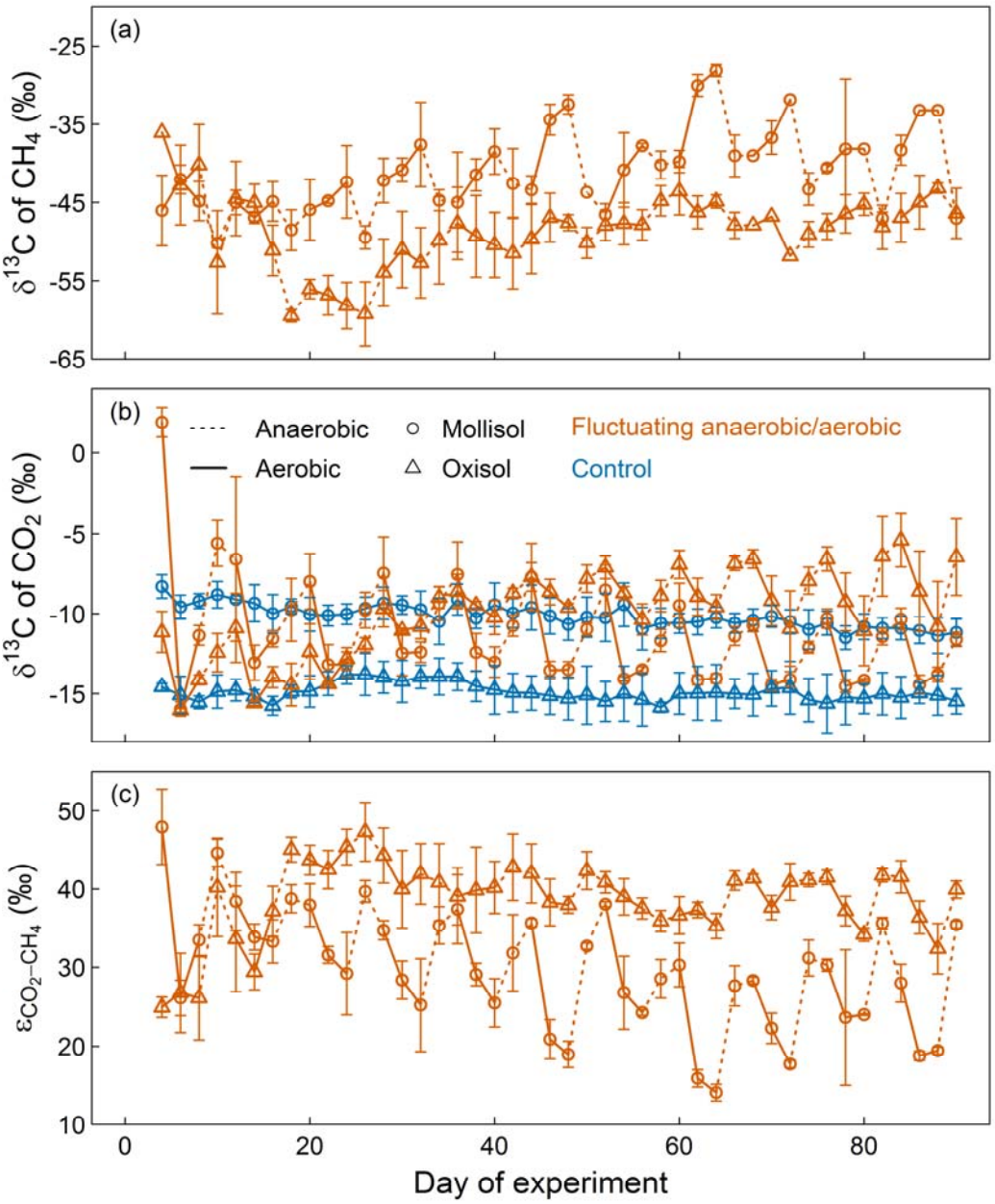
(a)
-25
-35
-45
-55
-65
δ13C of CH4 (‰)
(b)
Anaerobic
Aerobic
Mollisol
Oxisol
Fluctuating anaerobic/aerobic
Control
0
-5
-10
-15
δ13C of CO2 (‰)
(c)
50
40
30
20
10
εCO2–CH4 (‰)
0
20
40
60
80
Day of experiment

Fig. 2

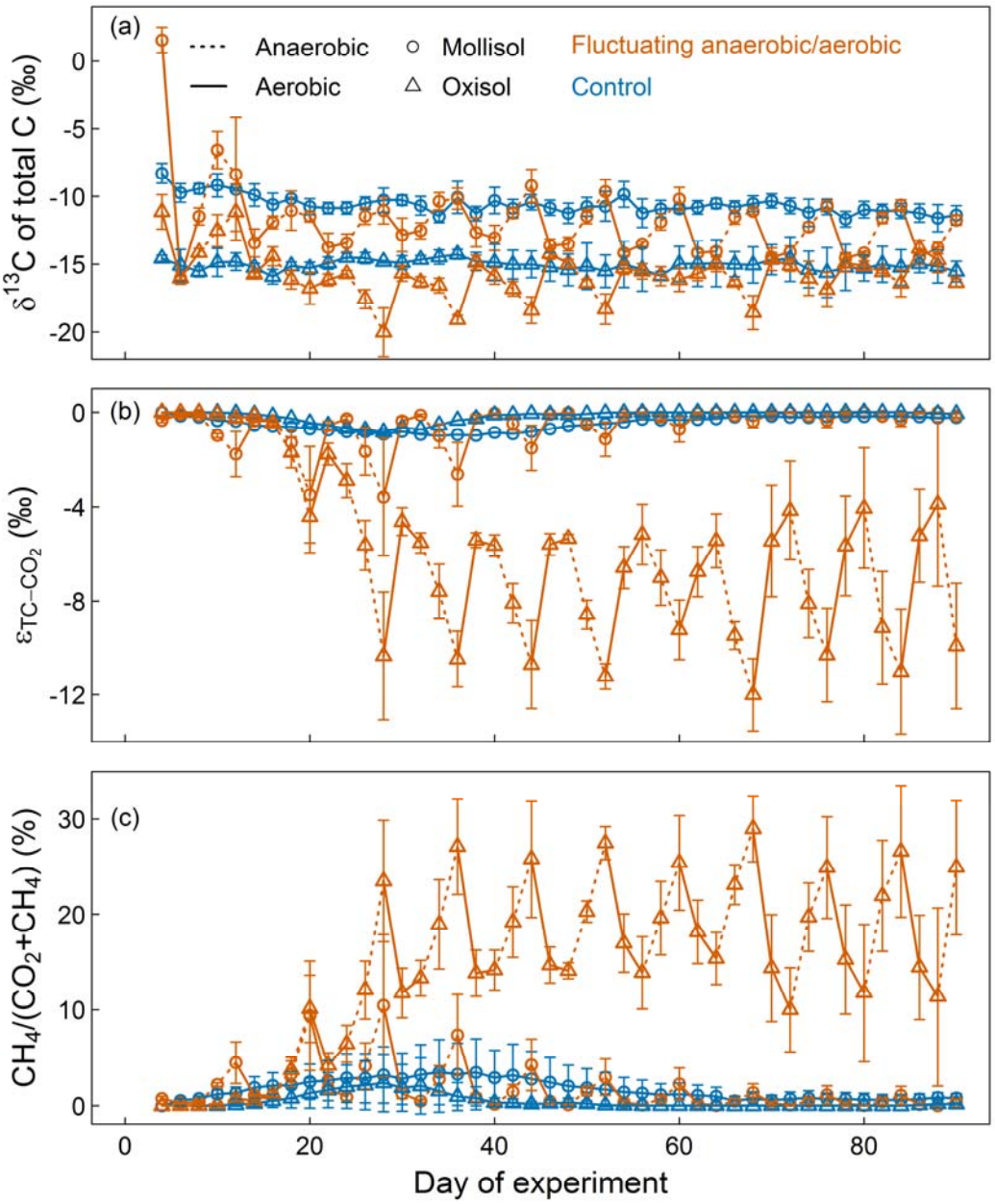
(a)
Anaerobic
Aerobic
Mollisol
Oxisol
Fluctuating anaerobic/aerobic
Control
δ13C of total C (‰)
0
-5
-10
-15
-20
(b)
εTC–CO2 (‰)
0
-4
-8
-12
(c)
CH4/(CO2+CH4) (%)
30
20
10
0
0
20
40
60
80
Day of experiment

Fig. 3

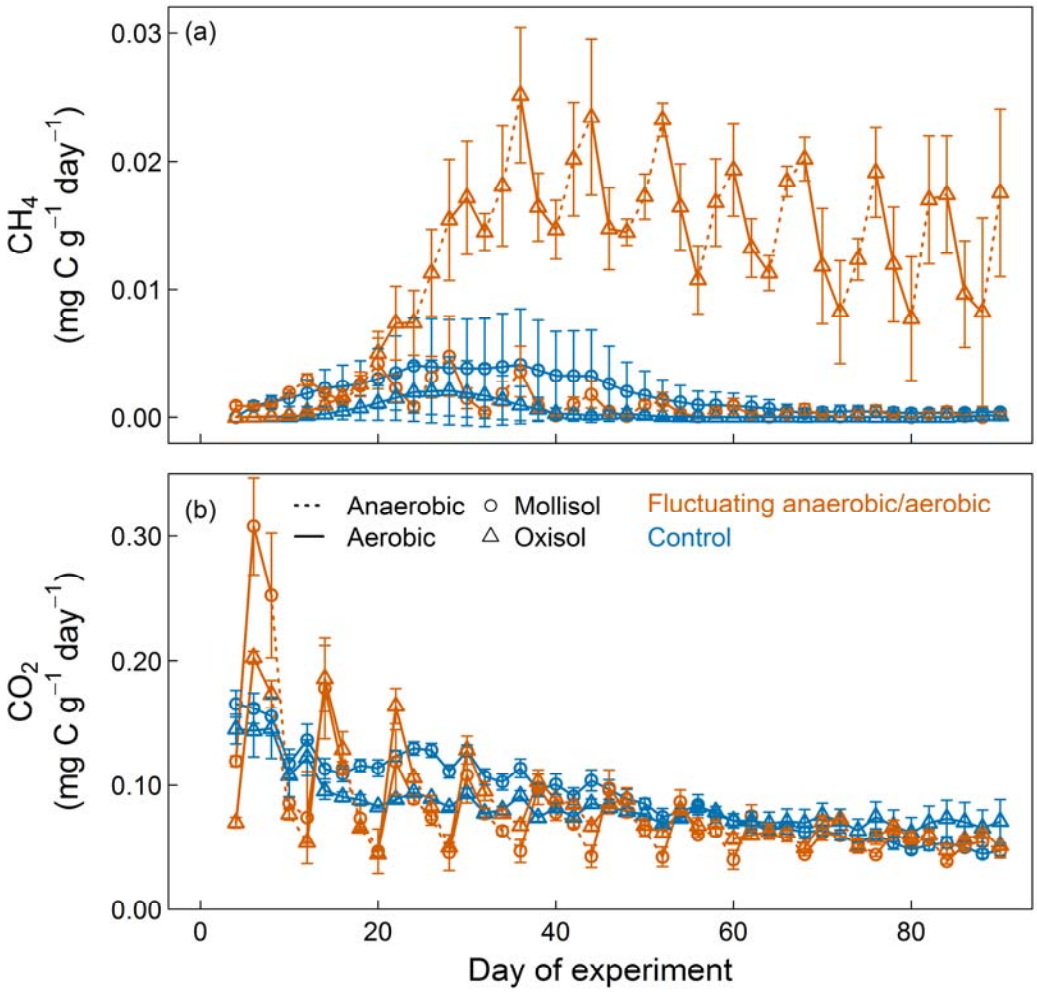
(a)
0.03
0.02
0.01
0.00
CH4
(mg C g−1 day−1)
(b)
Anaerobic
Aerobic
Mollisol
Oxisol
Fluctuating anaerobic/aerobic
Control
0.30
0.20
0.10
0.00
CO2
(mg C g−1 day−1)
0
20
40
60
80
Day of experiment

Fig. 4