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The fate of carbon utilized by the subterranean termite *Reticulitermes flavipes*

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Abstract. Subterranean termites are ecosystem engineers that modulate the flow of carbon from dead wood to the atmosphere and soil, yet their contributions to the latter pool are largely unaccounted for in carbon cycling models. The fate of C from wood utilized by *Reticulitermes flavipes* (Kollar) was determined using a reductionist design in a closed system with $\delta^{13}C$ labeled wood as a stable isotope tracer. The percentage of wood-based carbon in termite respiratory gases, tissues, and organic deposits (frass and construction materials) was measured for five colonies to budget wood-C mass distributed into metabolic and behavioral pathways during a 160-h incubation period. We found that termites emitted 42% of the C from wood as gas (largely as carbon dioxide), returned 40% to the environment as organic deposits (frass and construction materials), and retained 18% in their tissues (whole alimentary tracts and de-gutted bodies). Our findings affirm that termites are a source of greenhouse gases but are also ecosystem engineers that return approximately half the C from dead wood as organic deposits into their surrounding environment.

Key words: carbon cycling; closed chamber; eastern subterranean termite; free-air carbon dioxide enrichment (FACE); mass balance; methane; wood decomposition.

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INTRODUCTION

The sequestration and release of carbon (C) from natural processes is a complex and critical component of climate change models that need quantifiable information on C flows to mitigate the complexities inherent in those models. Temperate forest ecosystems are an important carbon sink in the context of the global carbon budget, occupying 25% of the world's forest cover with over one-third of forest C stored in wood biomass (Dixon et al. 1994, Pan et al. 2011). Temperate forest dead wood, compared to leaf litter and living components, is an understudied C-stock despite decomposition influences on carbon pathways (Cornwell et al. 2009, Pan et al. 2011). Wood decomposition is mediated by a guild of organisms that includes fungi, bacteria, and a diverse assemblage of saproxylic invertebrates that drive fragmentation and consumption (Harmon et al. 1986, Hanula 1996, Ulyshen and Wagner 2013). Current biogeochemical C models lack quantitative data on invertebrate-mediated C flows from wood to the atmosphere, living biomass, and soil pools of forest systems (Cornwell et al. 2009, Ulyshen and Wagner 2013). We postulate that wood-feeding subterranean termites merit attention in modeling global C, because of their contributions to greenhouse gas emissions and

additions to forest soils (Sugimoto et al. 2000, Myer and Forschler 2019).

Subterranean termites in the genus *Reticulitermes* are abundant throughout their Holarctic distributional range and are the invertebrates most efficient at reducing dead wood mass, playing a prominent role in forest C cycles (Martin 1983, Bourguignon et al. 2016). Subterranean termites extensively utilize cellulose, hemicellulose, and acidic sugars with the aid of gut symbionts, in contrast to many saproxylic insects that pass large amounts of undigested wood through their digestive tracts (Martin 1983, Katsumata et al. 2007, Raychoudhury et al. 2013). Fermentative processes by termite hindgut microbiota yield acetate and other short-chain fatty acids absorbed by the termite, and various other pathways also aid in wood digestion throughout the alimentary tract (Raychoudhury et al. 2013, Brune 2014). The role of *Reticulitermes* in soil C cycles has received less attention although they can increase soil C in a microcosm (Neupane et al. (2015) and shelter tubes contain higher %C than soil (Myer and Forschler 2019). There are scant data on the structure and maintenance of subterranean termite belowground biogenic structures but it is likely they incorporate a variety of C sources into the soil matrix through belowground construction activities and defecation (Ebeling 1968, Myer and Forschler 2019). The ubiquity and abundance of subterranean termites in temperate forests highlights the need for quantitative information on their role in C flows to better understand carbon cycle dynamics in these ecosystems.

Stable isotopes are a useful tool for tracing C flows through arthropod systems and can involve the labeling of natural food sources (IAEA 2009). The labeled food source used for this study originated from the U.S. Department of Energy Free Air CO_2 Enrichment (FACE) project at Duke Forest (Orange County, NC, USA) that grew *Pinus taeda* (loblolly pine) trees under long-term elevated CO_2 fumigation, resulting in a depleted $\delta^{13}C$ signature in the tree tissues (Andrews et al. 1999, Finzi et al. 2001). The objective of this study was to use FACE wood as a stable isotope tracer to determine the fate of wood-based C utilized by *Reticulitermes flavipes* (Kollar) using a reductionist, closed chamber system with only termites and wood.

Methods

Experimental design

Our labeled C source ("FACE wood") consisted of wood cut from a single loblolly pine log (93 cm length, 14.6 cm top diameter, 15.9 cm bottom diameter) that originated from the Duke Forest FACE site. We verified that FACE wood had the depleted isotope signature ($\delta^{13}C$ values, expressed in per milles or parts in one thousand, ranged between −41‰ and −39‰) before use in the experiment (see Appendix S1 for processing details). Wood pieces without the isotope label ("ambient wood") represented the isotopic baseline and were obtained from a single loblolly pine tree grown in ambient conditions ($\delta^{13}C$ values ranged from −29‰ to −24‰).

Each experimental unit was a pair of glass vessels (hereafter referred to as "vessel pair") that contained termites collected from the same colony (Table 1): one with 1500 termites and FACE wood, and the other with 1500 termites and ambient wood (n = 5). Ten gas samples were collected from each vessel over a 160-h incubation period to estimate CO_2 and CH_4 mass at each time-point and calculate accumulation rates. The substrates (solid products) collected included alimentary tracts, bodies, wood, and organic deposits (frass and construction materials). The total mass of the separate substrates was recorded at the end of incubation and respective $\delta^{13}C$ and % C_{total} (C mass/total sample mass) values analyzed to account for wood-based carbon in the various termite products.

Vessel components

The glass vessels (2 L) were hand-made at the University of Georgia Glass Shop, with the interior bottom sand blasted to provide a textured walking surface for the termites. The base and lid fit together at a 1-cm flange, cushioned with a circular rubber gasket (outer diameter 176 mm, inner diameter 148 mm) cut from a 4-mm-thick industrial neoprene rubber sheet (Fig. 1). Lids had two raised sampling ports (inner diameter 24 mm), one fitted with a fold-over white rubber septum (Precision Seal) for gas sampling and the other with a rubber stopper (one-hole, size 5, top: 27 mm, bottom: 23 mm) wired with an Adafruit MPL3115A2-I2C Barometric/Altitude/Temperature Sensor and made airtight with silicone

Table 1. Vessel set-up and take-down measurements.

Vessel pairs	Wood type	Termite weight (g)	Wood H_2O (%)	Termite survival (%)	Wood carbon loss (mg)	HS vol. (ml)
WH1	Ambient	4.95	32.2	94.2	232	2219
WH1	FACE	4.95	60.0	71.0	209	2034
WH2a	Ambient	4.35	32.7	95.2	227	1901
WH2a	FACE	4.35	42.7	92.8	155	1853
WH2b	Ambient	4.35	32.9	97.2	199	2134
WH2b	FACE	4.35	43.1	99.4	156	2024
WH3	Ambient	4.65	23.2	84.7	110	2047
WH3	FACE	4.65	26.8	82.5	86	2219
DW1	Ambient	5.10	21.0	92.0	43	2036
DW1	FACE	5.10	48.5	86.1	31	1895
OCM1	Ambient	4.65	27.4	93.7	230	1882
OCM1	FACE	4.65	32.2	93.8	0	2143
Mean		4.68	35.0	90.2	140	2032
SE		0.08	3.3	2.3	24.2	37.3

Notes: Each vessel pair consisted of two separate vessels containing 1500 termites from the same colony, provisioned with either FACE or ambient wood. The mean weight of 5 groups of 10 random termites per colony was used to estimate the mass of one termite, scaled up to estimate the mass of 1500 termites weighed, and set aside for each vessel. A representative value for termite density (0.97 g/mL), used for termite volume estimates, was determined using the water displacement method with 3.4 g of live termites and a drop of surfactant (Profoam Platinum). Pre-incubation wood water weight was calculated by: % Wood H_2O = (wet weight − dry weight)/wet weight × 100. Percent survivorship was determined by: % Survival = (number of live termites/1500) × 100. Wood mass loss in each vessel (pre-incubation dry weight − post-incubation dry weight) was adjusted with average wood mass loss of process blanks (n = 6; 303 ± 89 mg); carbon loss was calculated by: (wood mass loss) × (wood % C_{total}). Headspace volumes were calculated by: (HS vol) = vessel volume − (termite volume + wood volume).

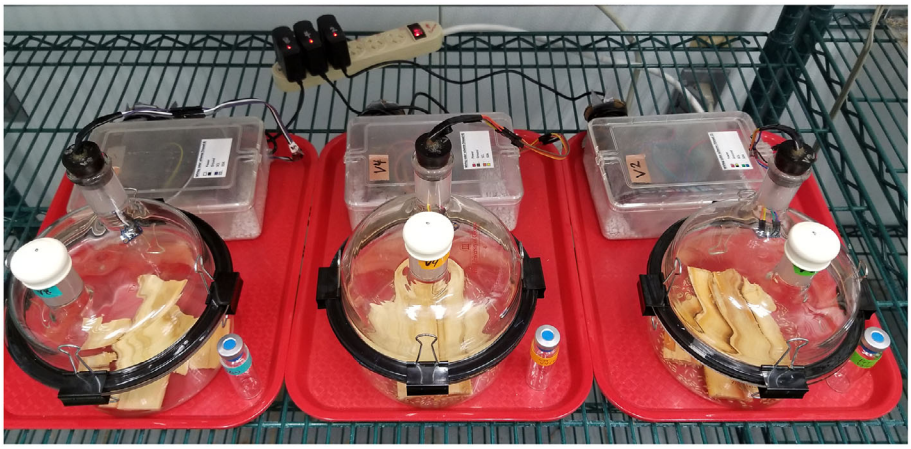

Fig. 1. Photograph of assembled vessels and pressure sensor units at the start of the gas sampling phase, just after the 24-h acclimation period and septa insertion.

sealant (Fig. 1). The sensor was suspended 1 cm below the port and connected to an Arduino® Mega 2560 board/ethernet shield and Adafruit DS3231 Precision Real-Time Clock Module programmed to record the date, time, and barometric pressure every 6 s (Fig. 1). Arduino boards were placed in plastic laminate boxes, nested in a hard outer box (Pioneer Plastics Rectangle Clear Plastic Box, 17 cm × 12 cm × 6 cm, l:w:h) containing 3 g of vermiculite (Fig. 1).

Preparation of termites and wood

Five *R. flavipes* colonies were collected from infested logs in Clarke County, Georgia (Whitehall Forest, WH; Dellwood Drive, DW; and Old Commerce Road, OCM; Table 1) using methods

described in Myer and Forschler (2019) and placed in colony-specific holding boxes (Pioneer Plastics Rectangle Clear Plastic Box, 17 cm × 12 cm × 6 cm, l:w:h) containing moistened pine wood planks (7.2 cm × 3.8 cm × 0.5 cm, l:w:h) with lids secured by Parafilm M (Bemis flexible packaging laboratory film) for no more than 1 month before incubation set-up.

Two groups of 1500 termites from each colony, consisting of a random sample of workers and soldiers with soldiers representing 1–2% per group, were placed in petri dishes (100 mm × 25 mm, polystyrene, Fisherbrand) lined with a moistened 9 cm #1 Whatman filter paper, sealed with Parafilm, and stored in the environmental chamber 48 h before the start of the incubation to void their alimentary tracts (Forschler 1996). Pairs of FACE and ambient wood pieces assigned to each vessel pair were oven-dried at 103°C for 24 h, transferred to a desiccation chamber to cool for 1 h, weighed (dry weight), submerged in plastic containers (15.5 cm diameter, 4 cm height) filled with deionized water for 24 h, then their volumes determined using the water displacement method in a 500-mL graduated cylinder. The wood sets were patted dry with paper towels, air-dried for 2 h, and weighed (wet weight) just prior to their placement in vessels. Process blanks consisting of vessel pairs with only FACE (n = 3) or ambient wood (n = 3) were incubated with termite vessels to control for wood mass loss due to these processing steps (Table 1).

Vessel incubation set-up

The arrangement of vessel pairs and blanks was randomized in the environmental chamber, with wood pieces laid out as a single layer in the open base of each vessel. The environmental chamber was maintained at 27°C, 78% humidity, and was kept in total darkness for the duration of the study. Fifteen workers from each petri dish of 1500 termites were de-gutted, and whole alimentary tracts and de-gutted bodies were placed in 20-mL glass scintillation vials sealed with an airtight, conical Polyseal cap and stored in the freezer (−15°C) until further processing to provide pre-incubation $\delta^{13}C$ and $\%C_{total}$ values. The remaining termites were transferred from their petri dishes to their assigned vessels (Table 1). Rubber gaskets between vessel lids and bases were sealed with a layer of DOW Corning high vacuum grease and secured with four medium-sized binder clips (Fig. 1). The gas sampling port was covered with Parafilm at the start of the 160-h incubation period.

Gas sampling

Termites have higher metabolic rates and emit more CO_2 and CH_4 when agitated or disturbed (Tyler 1986). Therefore, gas sampling was preceded by an acclimation phase when the gas sampling port was covered with parafilm for 24 h. Vessels were made airtight by replacing the parafilm with fold-over rubber septa (Fig. 1) to start the gas sampling phase in which headspace gas samples (10 mL) were drawn through each septum port at 10 time-points (24, 40, 48, 64, 72, 88, 96, 112, 120, and 136 h) using a SGE 10-mL gastight syringe pre-fitted with a Luer-lock™ push button lock valve (10MR-VLLMA-GT). The syringe was attached to a three-way stopcock and 24-gauge needle that was inserted into a Supel inert foil gas sampling bag (push lock valve, 2 L) filled with analytical grade N_2 to flush the syringe twice before each sample collection. The syringe was locked after flushing, and a 22-gauge needle was attached and inserted into the vessel septum. The plunger was incrementally adjusted to a 10 mL sample volume; the syringe was locked, removed from the septum, unlocked, and injected into a 20-mL borosilicate headspace vial fitted with standard blue butyl rubber stoppers and aluminum crimp seals that were evacuated and pre-filled with 10 mL of Zero Air (Airgas Part #AI Z300) before sampling. Collected gas samples were stored at room temperature until analysis.

Disassembly

Septa were replaced with Parafilm after the last gas samples for a 24-h re-acclimation phase to allow the termites to recuperate from hypoxia prior to disassembly, which completed the 160-h incubation period. The remaining termites were counted to determine survivorship at the end of the experimental period, and 20 workers from each vessel were dissected to obtain post-incubation alimentary tracts and de-gutted bodies. Frass and construction materials on the wood and vessel surfaces were dislodged and placed in scintillation vials (Fig. 2). Wood planks were oven-dried (103°C)

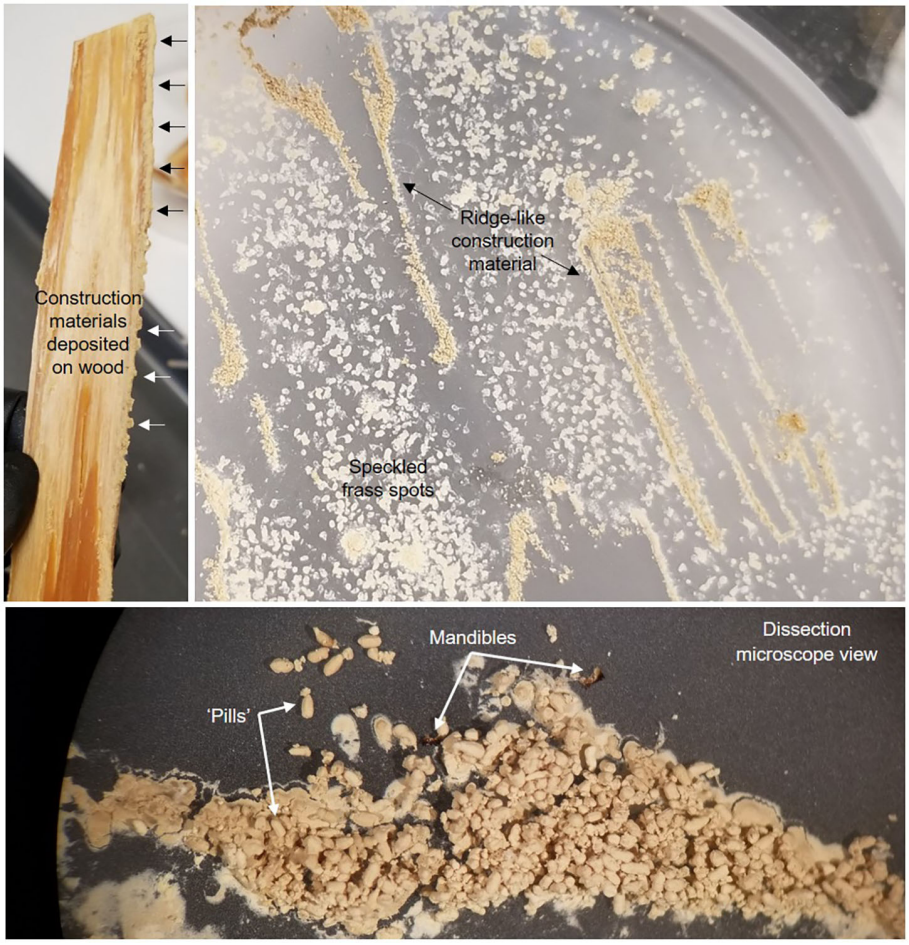

Fig. 2. Organic deposits collected during vessel disassembly, including construction materials along wood (CON; upper left), and ridge-like construction material (RID) & frass spots (FRA) adhered to bases (upper right). Masticated wood particles shaped into "pills" and mandibles were present in RID of vessels with drier wood (<30% moisture). See Myer and Forschler (2019) for more detailed definitions.

for 24 h, transferred to a desiccator for 1 h, and weighed. Cross-grain sections (1 cm) from the end of each plank were homogenized to a fine powder using a ball mill, transferred to glass scintillation vials, and stored in a freezer at −15°C until further processing.

Chemical analysis

Concentrations (ppm) and $\delta^{13}C$ signatures of CH_4 and CO_2 were determined by cavity ring-down spectroscopy (CRDS) using a Picarro G2201-i Analyzer equipped with Small Sample Introduction Module 2 (Picarro, Inc. Santa Clara, CA, USA). A 2.5-mL gastight syringe with a push/pull lock valve (SGE syringe: 2.5MDR-VLL-GT) attached to an 18-gauge needle was flushed three times in an open N_2 stream, inserted into the sample vial to withdraw 0.75 mL and locked. The syringe was unlocked, and the plunger pushed to 0.5 mL, and locked. The needle tip was inserted into an open stream of Zero Air, the plunger pulled to 2.0 mL, and the syringe unlocked for 2 s and locked to fill the 2.0 mL space for a dilution factor of 1:3. The syringe was attached to the Picarro injection module for analysis in Dual CO_2/CH_4 mode (CH_4 High Range operational range: 100–500 ppm). The machine further diluted the injected gas by a factor of 10; therefore, all our samples were diluted by a factor of 80 [final dilution factor = 2 (vial) × 4

(syringe) × 10 (Picarro)]. See Appendix S2 gas sampling and analytical method accuracy testing using known standards.

Solid samples were freeze-dried for 16 h, crushed with a clean glass rod, encapsulated, and weighed (0.6–1.8 mg) using the Stable Isotope Ecology Laboratory (SIEL, University of Georgia Center for Applied Isotope Studies) protocol for preparing organic matter for analysis (https://cais.uga.edu/stable-isotope-ecology-laboratory). The $\%C_{total}$ (C mass/total sample mass) and $\delta^{13}C$ signatures of all the solid samples were determined via a C/N elemental analyzer coupled to an isotope-ratio mass spectrometer (IRMS) by the SIEL. The remaining post-incubation de-gutted bodies, frass, and construction material stored in airtight vials were re-dried in an oven (55°C for 2 h) and weighed for total dry mass.

Calculations of gas and wood-based carbon flows

The Ideal Gas Law (Eq. 1) was used to determine total gas content per vessel headspace (Table 1). Moles were converted from ppm to mass using dimensional analysis to estimate the mass of each gas at each time-point and CO_2 and CH_4 accumulation rates.

$$PV = nRT \quad (1)$$

where P is the barometric pressure (atm) in each vessel measured by a sensor prior to each sample withdrawal, V is the vessel headspace volume (L); n is the total moles of gas; R is the gas law constant (0.08206 $L{\cdot}atm{\cdot}K^{-1}{\cdot}mol^{-1}$), and T is the temperature of the environmental room (27°C or 300.15 K).

Isotope ratios (Eq. 2) were expressed in the delta notation (Craig 1953, 1957). The percentage of carbon atoms in each product of each vessel pair that came from provided wood ($\%C_{wood\text{-}based}$; Eq. 3) was expressed as a ratio of differences between $\delta^{13}C$ values of each product divided by $\delta^{13}C$ values of each source, using methods adapted from IAEA (2009).

$$\delta^{13}C_{sample}\ (‰) = \left[\frac{\frac{^{13}C}{^{12}C_{sample}} - \frac{^{13}C}{^{12}C_{standard}}}{\frac{^{13}C}{^{12}C_{standard}}}\right] \times 1000 \quad (2)$$

where the standard is Pee Dee Belemnite (PDB); $\frac{^{13}C}{^{12}C}$ is the IRMS-derived ratio of C^{13} to C^{12}; and the unit expressed is per mille, or parts in one thousand (‰).

$$\%C_{wood-based} = \left[\frac{\delta^{13}C_{ProductF} - \delta^{13}C_{ProductA}}{\delta^{13}C_{SourceF} - \delta^{13}C_{SourceA}}\right] \times 100 \quad (3)$$

where $\%C_{wood\text{-}based}$ is the percent of carbon atoms in the product derived from wood; $\delta^{13}C_{(ProductF)}$ and $\delta^{13}C_{(ProductA)}$ are delta values of termite colony products (frass, CO_2, etc.) in each vessel pair with either FACE or ambient wood (respectively); $\delta^{13}C_{(SourceF)}$ and $\delta^{13}C_{(SourceA)}$ represent the delta values of the pre-incubation FACE and ambient wood in each vessel (respectively); and the unit expressed is percent (%).

Total carbon mass of the various solid products per vessel (C_{mass}) was calculated by multiplying the total dry mass of each product by its corresponding $\%C_{total}$; wood-based C mass in each product per vessel was determined by multiplying C_{mass} by $\%C_{wood\text{-}based}$. All calculations were performed in R version 3.5.1 (R Development Core Team 2021); see code file and corresponding Metadata in Data S1.

Results

Gas accumulation and $\delta^{13}C$

Simple linear models by treatment provided accumulation rates of 3.571 mg CO_2/h and 0.01598 mg CH_4/h in vessels containing termites (Fig. 3). Maximum percent concentrations of CO_2 and CH_4 at the end of the gas sampling phase were 17% and 0.28% (respectively), with similar CH_4 concentrations within vessel pairs (Appendix S3: Fig. S1). Wood-based C mass was estimated for both gases with averages that increased during the gas sampling phase from 10 mg to 71 mg for CO_2, and 0.45 mg to 0.89 mg for CH_4 (Table 2, Appendix S3: Table S1). Vessel pairs provided CO_2 $\delta^{13}C$ values separated distinctly by treatment, whereas CH_4 $\delta^{13}C$ values provided more overlap (Fig. 4). All $\delta^{13}C$ values indicated a ≥5‰ difference between paired vessels, except CH_4 $\delta^{13}C$ values for the vessel pair with colony WH3 (Fig. 4). Levels of CO_2 and CH_4 in gas samples from process blanks (wood only vessels) were below the operational detection limits of the Picarro (Table 2, Appendix S3: Table S1).

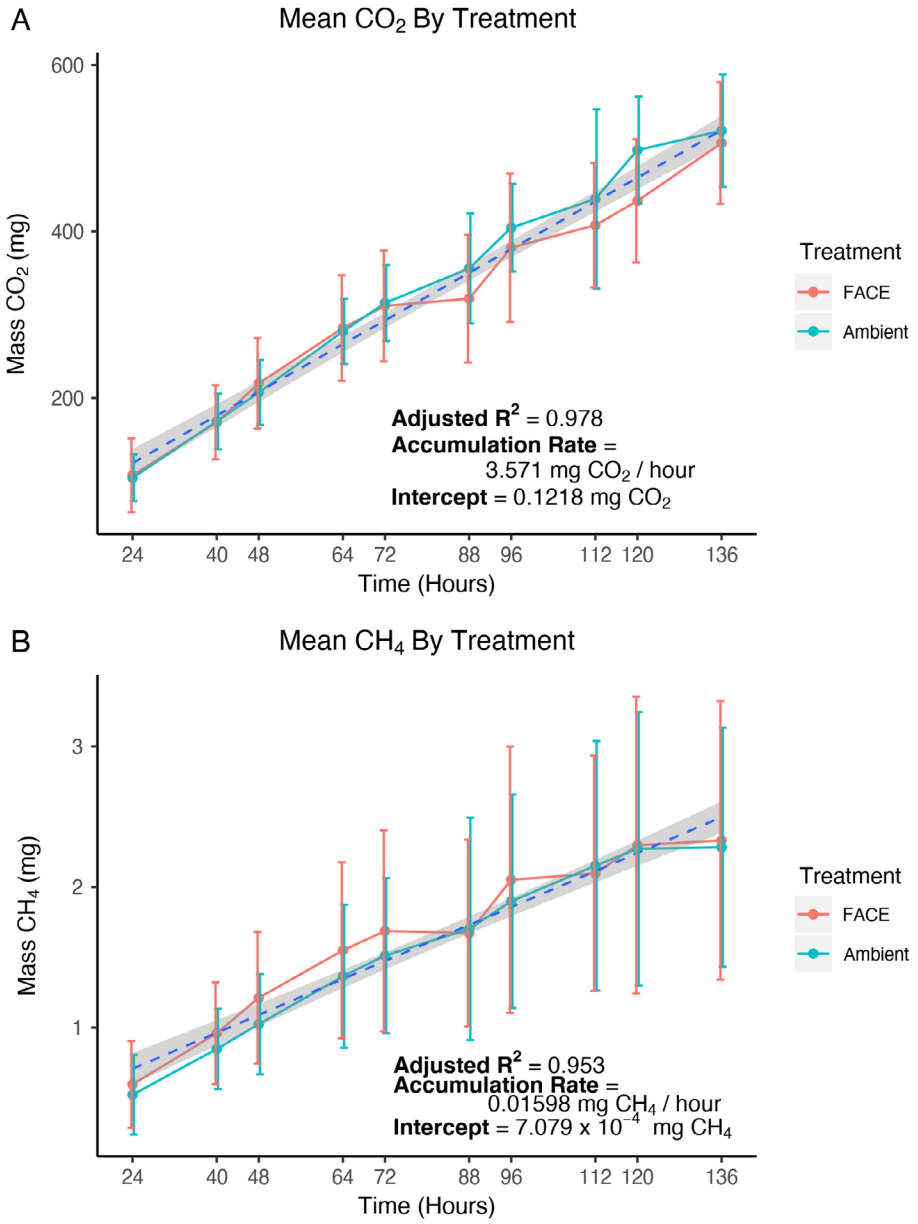

Fig. 3. Average mass ± standard error (n = 6 vessel pairs) of CO_2 (A) and CH_4 (B) over the gas sampling phase (solid lines) fitted to a simple linear model (dashed line with shaded area denoting standard error).

Solid product C mass and $\delta^{13}C$

Pre-incubation ambient wood and termite tissues provided ^{13}C means of approximately 26.7‰, whereas FACE wood had a more depleted $\delta^{13}C$ mean of −39.9‰ (Fig. 5). The % $C_{wood\text{-}based}$ calculated from $\delta^{13}C$ values of the post-incubation substrates were as follows: 98.6% in wood, 83.8% in ridge-like construction materials, 83.3% in construction materials dislodged from wood, 82.1% in frass, 22.3% in guts, and 4.2% in de-gutted bodies after 160 h (Fig. 5). The additional percentage (1.4%) of carbon on post-incubation wood (Fig. 5) can be attributed to termite frass deposited on the wood (Appendix S3: Fig. S2). The average wood-based C mass in the vessels was represented by 66 mg in

Table 2. Wood-based C mass (mg) in CO_2 over gas sampling phase.

Vessel pair	24 h	40 h	48 h	64 h	72 h	88 h	96 h	112 h	120 h	136 h
DW1	2	9	11	16	17	19	21	26	27	39
OCM1	9	20	27	42	45	52	66	69	80	96
WH1	19	27	36	48	56	49	72	73	79	–
WH2a	15	27	32	43	51	59	67	77	78	81
WH2b	11	19	27	41	43	57	64	73	74	80
WH3	5	12	15	21	26	30	35	44	49	57
Blanks	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD
Mean	10	19	25	35	40	44	54	60	65	71
SE	3	3	4	5	6	7	9	8	9	9

Notes: Estimates of wood-based carbon mass (mg) in CO_2 measured at each time-point (hours), calculated by: (Carbon mass in CO_2) × (% $C_{wood\text{-}based}$). Carbon mass in CO_2 was determined using dimensional analysis with molecular weights of both $^{12}CO_2$ and $^{13}CO_2$ and ppm values for each vessel and time-point. Vessel pairs were averaged and listed as representative values. The vessel with colony WH1 and FACE wood was re-acclimated early (96 h) when all the termites were immobile and adhered to the vessel bases due to condensation. Blanks (vessels with wood only) provided by CO_2 concentrations were below the operational limits of the Picarro, and thus, lacked accurate $\delta^{13}C$ values needed to estimate % $C_{wood\text{-}based}$.

construction material and frass and 30 mg in guts and bodies (Table 3). To investigate the comparatively low level of wood-based C in the body tissues, we conducted a separate bioassay with non-airtight boxes (Pioneer Plastics® Rectangle Clear Plastic Box, 17 cm × 12 cm × 6 cm, l:w:h) to estimate the assimilation rate of wood-based C in termite tissues over a longer time-frame of 18 d (see Appendix S4 for details). The % $C_{wood\text{-}based}$ increased at a rate of 2.23% per day in guts and 0.88% per day in de-gutted bodies (Appendix S4: Fig. S1).

Closed chamber C budget

An average of 140 mg of wood-based C loss was mediated by termite activity during the 160 h incubation period (Table 1), which was 0.98% (140/14,300 mg) of the average C mass in wood provided to termites (estimated in each vessel by: pre-incubation dry weight × %C) Approximately 71 mg of wood-based C in CO_2 and 0.9 mg of wood-based C in CH_4 were present by 160 h (Table 2). The mass of solid samples accounted for 95 mg of wood-based C (Fig. 2, Table 3). Overall, our averaged data accounted for all wood-based C in the various termite products by the end of the experiment (Fig. 6, Tables 2, 3, Appendix S3: Table S1).

Discussion

This experiment, the first to directly measure partitioning of wood-C into termite tissues, CO2, CH4, and organic deposits using a closed system, accounted for C flow from wood through termites. Approximately half (42%) of the C from wood was emitted as CO2 during the 160-h incubation, with an additional 40% in organic deposits (construction materials, frass), 18% in termite tissues, and <1% in CH4 (Fig. 6, Tables 2, 3, Appendix S3: Table S1). These findings validate the inference by Khalil et al. (1990) that "the large fraction of carbon goes in roughly equal amounts into the fecal material and in the gas phase (mostly as CO2 and little CH4)" and provide a mechanistic basis for partitioning the fate of consumed wood C. The post-incubation $\delta^{13}C$ values indicated the presence varying amounts of $\%C_{wood\text{-}based}$ between the solid products (Fig. 5), which highlights the necessity of separating these different wood-based carbon pools (Figs. 2, 6).

Gas accumulation rates measured from the vessels support previous termite studies that used closed chamber systems. Our linear model CH_4 accumulation rate of 21.3×10^{-8} mol CH_4/g fresh termite/h (converted from 0.01598 mg CH_4/h; Table 1, Fig. 3B) was within the range of previous reports with *Reticulitermes* colonies, which varied between below detection limits to 33.1×10^{-8} mol CH_4/g fresh termite/h (Odelson and Breznak 1983, Wheeler et al. 1996, Sugimoto et al. 1998). This intercolonial variability was also observed in our data set and increased over time (Appendix S3: Fig. S1B). Our mean CO_2 accumulation rate of 0.414 μL/mg fresh termite/h (converted from 3.571 mg CO_2/h; Table 1, Fig. 3A) was comparable to Wagner et al. (2012)

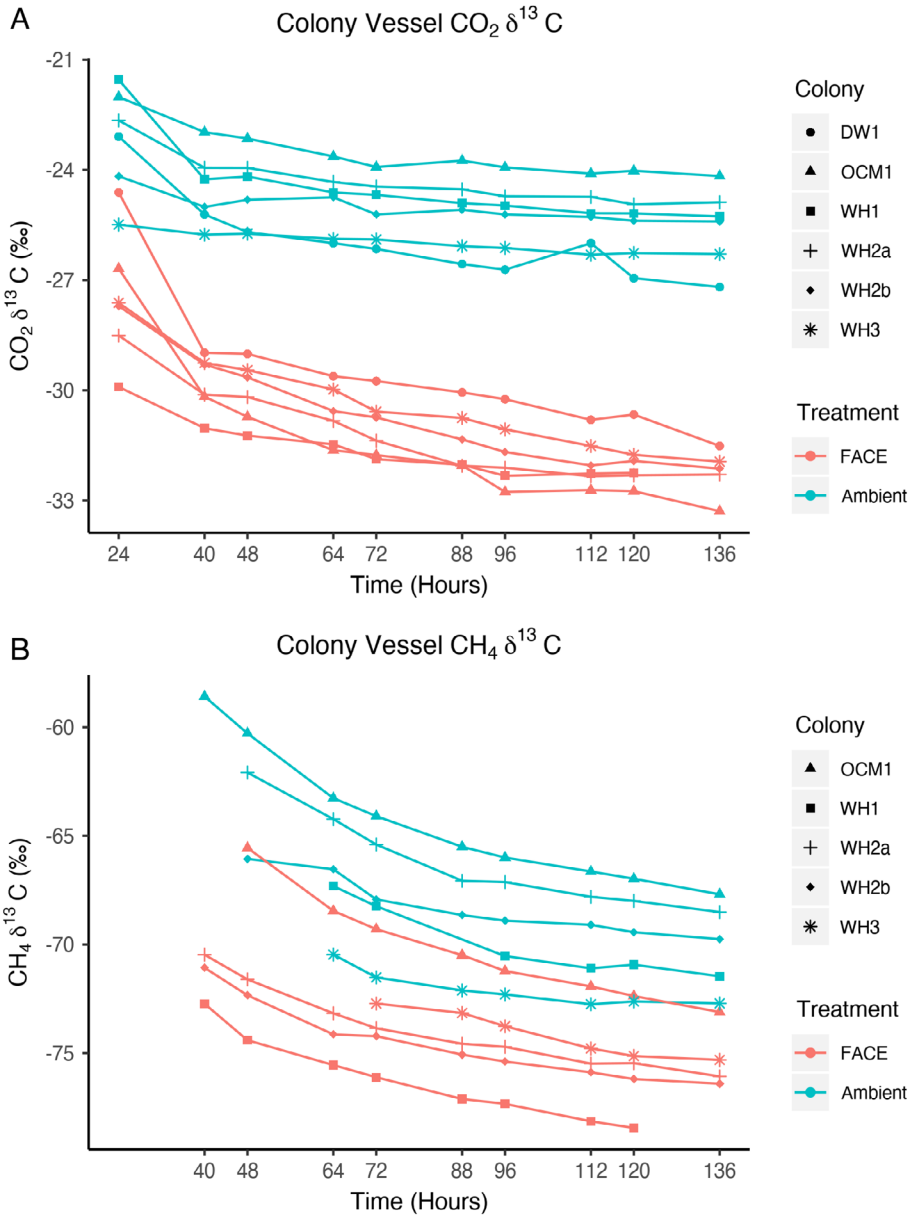

Fig. 4. (A) CO_2 δ^{13}C (‰) and (B) CH_4 δ^{13}C (‰) in each vessel pair over time. Missing values in (B) were from time-points that provided CH_4 concentrations below the CH_4 high range (HR) mode operational limits of the Picarro. Colony DW1 was the vessel pair with concentrations low enough to be measured under HP mode and ranged between −84‰ and −78‰.

(0.397 µL/mg fresh termite/h) who used groups of 10 *R. virginicus* workers but lower than the 0.544 µL CO_2/mg fresh termite/h reported by Shelton and Appel (2001*a*). The latter authors used respirometry methods that exposed individual *R. flavipes* workers to streams of air, a method later found to cause large bursts of CO_2 in isolated subterranean termites that would otherwise release CO_2 continuously under higher humidity (Shelton and Appel 2001*a*, Sláma et al. 2007, *b*). Survivorship in the vessels (mean 90.2% ± 2.3, Table 1) shows that termites can be

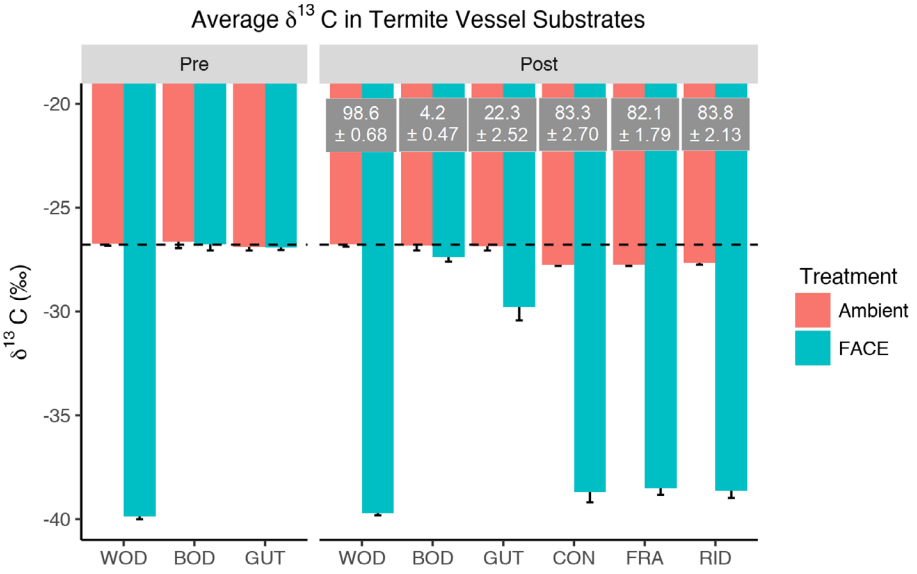

Fig. 5. Mean ± standard error of $\delta^{13}C$ values by solid products (substrates) for pre- (Pre) and post-incubation (Post) exposure of termites with wood after 160 h (n = 6 vessel pairs). Substrates: wood (WOD); de-gutted bodies (BOD); whole alimentary tracts (GUT); construction materials deposited on wood (CON); frass (FRA); and ridge-like construction materials deposited on vessel bases (RID). The dashed baseline shows mean $\delta^{13}C$ of pre-incubation ambient wood, and mean ± SE of $\%C_{wood\text{-}based}$ (calculated by each vessel pair) is listed in gray boxes.

Table 3. Wood-based C mass (mg) in solid products after 160-h incubation period.

Vessel	Treatment	WdCon	Ridge	Frass	Guts	Bodies	Total
DW1	Ambient	28	7	1	15	10	61
OCM1	Ambient	21	45	24	22	15	127
WH1	Ambient	44	47	12	8	15	126
WH2a	Ambient	46	50	14	7	7	124
WH2b	Ambient	27	42	23	13	15	120
WH3	Ambient	6	18	8	23	25	80
DW1	FACE	10	23	7	22	10	72
OCM1	FACE	56	12	4	22	14	108
WH1	FACE	29	14	9	6	15	73
WH2a	FACE	22	16	3	5	7	53
WH2b	FACE	14	43	7	17	17	98
WH3	FACE	15	25	7	22	25	94
Mean		27	29	10	15	15	95
SE		4	5	2	2	2	8

Notes: Wood-based C mass in construction material collected off wood (WdCon), ridge-like construction material (Ridge), and frass were calculated by: $C_{mass} \times (\%\ C_{wood\text{-}based})$. Wood-based C mass in tissue samples was calculated using the same formula based on dry weights of alimentary tracts and de-gutted bodies of 20 termites (adjusted by a factor of 75 = 1500 total/20 sampled).

maintained in high CO_2 conditions, and our CO_2 and CH_4 concentrations at the end of gas sampling (14% and 0.19%, respectively) were higher than previous reports with fewer termites and shorter sampling periods (Appendix S3: Fig. S1A, Anderson and Ultsch 1987, Wheeler et al. 1996, Tasaki et al. 2020).

Termites are best known for their contribution to carbon cycling by the emission of greenhouse gases, estimated to produce 2–22 Tg of CH_4 per

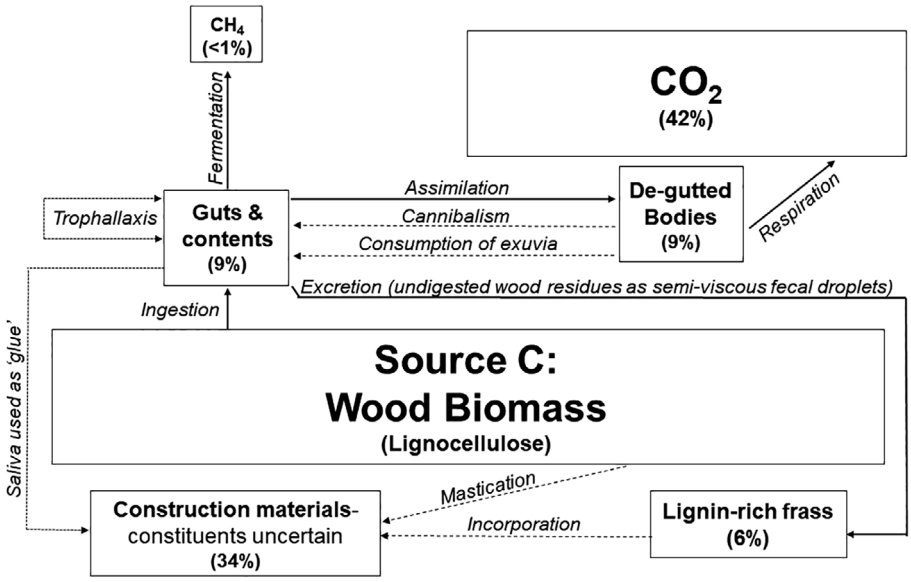

Fig. 6. Theoretical flowchart linking wood-based carbon pools (boxes) in the 160-h incubation period. Processes directing C flows that were measured (solid) and unmeasured (dashed) in our system are shown by arrows.

year (<5% of global emissions) with variations by taxa and geographic location (Sanderson 1996, Sugimoto et al. 2000). Fermentation by termite gut microbiota yields acetate and short-chain fatty acids in a process that produces CO_2 reduced to CH_4 by symbiotic methanogens (Raychoudhury et al. 2013, Brune 2014). The ranges of our CH_4 $\delta^{13}C$ values and accumulation rates may reflect natural variability between colonies, perhaps a result of different gut microbe assemblages that aid in wood digestion via complex and interconnected CO_2 and CH_4 pathways (Figs. 3B, 4B; Sugimoto et al. 1998, Brune 2014). This variability likely explains the greater overlap in CH_4 $\delta^{13}C$ values compared to CO_2. CH_4 $\delta^{13}C$ values provided by the vessel pair with colony WH3 lacked a ≥5‰ separation (Fig. 4B). *Agamomermis termitivoratus* n. sp., an endoparasitic nematode, was found during post-incubation dissections of termites from colony WH3 (Appendix S3: Fig. S3, Poinar et al. 2019), and likely hindered normal digestive processes in the infected termites.

Whitman and Forschler (2007) observed that only ~40% of *Reticulitermes* feeding events were direct consumption of cellulose, with the remainder being indirect feeding via trophallaxis. This "eusocial stomach," cannibalism, and consumption of exuvia adds layers of complexity to C flow within termite tissues (Fig. 6), with trophic effects that inevitably affect $\delta^{13}C$ (Gannes et al. 1997, Sun and Zhou 2013). Wood-C loss was lower in the FACE treatments in each vessel pair (Table 1), suggesting a lower palatability of FACE wood that has a higher density of 0.59 g/cm^3 compared to 0.46 g/cm^3 in ambient wood (Trettin, *unpublished data*). Elevated CO_2 conditions have been reported to change physical and anatomical properties of stemwood, yet have no effect on C:N ratio or the content of cellulose, lignin, and other organic compounds (Runion et al. 1999, Atwell et al. 2003, Kostiainen et al. 2009). Therefore, the assumption behind our calculations for % $C_{wood\text{-}based}$ (Formula 3) was that all C flows were the same between containers with either FACE or ambient wood in this reductionist study.

We provide evidence that termites have a dual role as a source of greenhouse gases and as an ecosystem engineer that returns approximately half the C from dead wood as organic deposits into their surrounding environment (Fig. 6). The contribution of *Reticulitermes* to gas emissions in a tallgrass prairie was previously investigated

using traditional soil flux chambers, but the results were confounded by the heterogeneous distribution of termites and their interactions with other soil fauna (Brown et al. 2008, Konemann et al. 2016). We intentionally excluded soil in a closed system to simplify the task of budgeting C mass loss and, in the end, accounted for all C in the system (Fig. 6, Tables 2, 3, Appendix S3: Table S1). Nauer et al. (2018) described tropical termite mounds as "biofilters" that oxidize half of termite CH_4 accumulation prior to atmospheric emission. A similar function may be performed within the soil-bound galleries of *Reticulitermes*. Accordingly, our gas release relate rates reflect gross CH_4 production and would overestimate the net release to the atmosphere from the soil or log surface.

Termites contribute to soil carbon stores by constructing biogenic structures presumed to be a mixture of soil, frass (feces), masticated wood particles, and saliva (Ebeling 1968). Their frass-lined biogenic structures harbor an unique assemblage of microorganisms, and similar to other biological soil aggregates, termite galleries are likely a stable soil organic carbon (SOC) pool unaccounted for in nutrient cycling models (Lal et al. 2015, Coleman et al. 2017). Forest biomes are a major terrestrial carbon sink, but there are many discrepancies among proposed hypotheses regarding the role of temperate forests in the global "missing carbon sink" (Myneni et al. 2001, Houghton 2007, Johnston et al. 2019). The greatest uncertainty in the C budget in the Northern Hemisphere is attributable to soil C, and many processes driving belowground C dynamics warrant further research before incorporation into forest C models (Pollierer et al. 2007, Chapin et al. 2009). Subterranean termites are abundant ecosystem engineers that modulate C flows between wood, soil, and the atmosphere, and our results suggest that *Reticulitermes* are likely drivers of belowground C processes (Fig. 6; Cornwell et al. 2009, Myer and Forschler 2019).

Contributions of *Reticulitermes* to carbon cycles should be considered in light of their eusociality, and attributes as soil fauna and wood degraders. This study took advantage of the unique isotopic properties of FACE wood to conduct a quantitative mass balance for wood-based C transformations in a closed chamber system with hundreds of termites and provides a framework to illustrate *Reticulitermes* modulation of wood-based C pathways (Fig. 6, Tables 1–3, Appendix S3: Table S1). We conclude that subterranean termites indeed contribute to greenhouse gases but also play an equivalent role depositing wood-based organic matter into the environment. Species richness, biomass consumption, and abundance of mound-building termites are well-documented and have been used to extrapolate the contribution of higher termites to gas emissions and soil properties in subtropical and tropical ecosystems (Bignell and Eggleton 2000, Sugimoto et al. 2000). Far less is known regarding subterranean termites common to temperate forests that lead a more spatially dispersed lifestyle (King and Spink 1969, Sanderson 1996, Sugimoto et al. 2000). Belowground processes are inherently difficult to measure, yet integration of tracer methods into field studies on the fate of wood-C utilized by subterranean termites is a promising direction for future research.

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Data Availability

Data and code are available in Data S1 and from Zenodo: https://doi.org/10.5281/zenodo.4729037

Supporting Information

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3872/full