



Consistent effects of eastern subterranean termites (*Reticulitermes flavipes*) on properties of a temperate forest soil



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ABSTRACT

Termites inhabit a large portion of land covered by temperate forests. Climate warming and urbanization will likely extend their range and increase their densities in these ecosystems but, compared to their tropical counterparts, little is known about their effects on soil properties and processes. If temperate termites have the strong ecosystem engineering effects of tropical termites, then knowledge of their ecology and impacts will be vital for predicting how temperate systems respond to environmental change. We investigated how feeding and tunneling by the eastern subterranean termite, *Reticulitermes flavipes*, affected wood decomposition and soil properties under decaying wood. Twelve laboratory microcosms filled with mineral soil and with wood blocks of four common temperate tree species, received *R. flavipes* soldiers and workers at field densities, with an additional five termite-free microcosms serving as controls. After 25 weeks, the effects of termites on wood mass loss, and on carbon and nitrogen dynamics, differed across tree species, yet their effects on soil properties were consistent regardless of wood type. Microbially-available carbon in soil was 20% higher with termites and soil moisture content 20% lower. Soil pH was more acid with termites and their effects on soil microbial biomass were positive but non-significant. These soil responses were consistent regardless of the wood species, suggesting that termite effects on soil are dictated largely by their activity within the soil matrix and not by their feeding rate on specific wood substrates. These results are among the first to quantify the effects of temperate forest termite activity on soil properties, demonstrating the potential for these termites to shape biogeochemical cycling and plant communities through their alteration of the soil environment.

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1. Introduction

Termites are ecosystem engineers, transforming the structure and functioning of grasslands, forests and savannah (Black and Okwakol, 1997; Dangerfield et al., 1998; Jouquet et al., 2011). The majority of research on termites has focused on tropical and subtropical systems, and commonly on species that form the charismatic nest mounds (Lavelle, 1984; King et al., 2013). Much less attention has been paid to the engineering effects of temperate forest termites, which do not form mounds but primarily nest in subterranean galleries and feed within dead and decaying wood (Nobre et al., 2009; King et al., 2013). The activities of these cryptic

organisms accelerate the breakdown rate of woody material and hence the rate at which organic material is incorporated into the soil (Kitchell et al., 1979; Kirker et al., 2012). Like other invertebrate macrofauna engineers, such as ants and earthworms, their tunneling activities can alter soil drainage (Léonard and Rajot, 2001; Léonard et al., 2004; Turner, 2006; Mettrop et al., 2013). Yet it is virtually unknown as to how such changes in soil inputs and structure translate to changes in soil microbial biomass, carbon (C) availability, pH and moisture (Maynard et al., 2015). That is, soil properties that strongly influence biogeochemical process rates and plant communities.

Estimates of temperate forest termite abundances are few, primarily because these eusocial insects are largely missed by most soil fauna sampling regimes because they do not target colonies or dead wood (Fierer et al., 2009). Those estimates we do have suggest that – along with ants – termites can be the most abundant invertebrate macrofauna in temperate forest floors (King et al., 2013). In some warm temperate regions their biomass can even

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approach that of many tropical termites (Gentry and Whitford, 1982; Vargo and Husseneder, 2009). Although less abundant as one moves toward the poles, subterranean termite species such as *Reticulitermes flavipes* – native to eastern North American forests – have ranges that extend far from the Equator (to at least 45° N). *Reticulitermes flavipes* is the most widely distributed termite species in North America and is found in the eastern region stretching from Ontario, Canada in the north to Florida, USA in the south (Austin et al., 2005). This species has also been reported as invasive in Austria, Germany, France, Bahamas, Uruguay and Chile (Evans et al., 2013). As a decomposer of woody material, native subterranean termites (*Reticulitermes* spp.) provide an important role in forest ecosystems (La Fage and Nutting, 1978; Ulyshen and Wagner, 2014). It is conceivable that factors such as climate warming will permit these species to increase their abundances and hence ecosystem effects at more northerly latitudes (Maynard et al., 2015). A paucity of data regarding their functional roles in temperate ecosystems currently prevents reliable projections of how changes in their abundances and geographic distributions will affect soil properties and hence forest structure and function.

We investigated how termite tunneling and feeding on wood affects soil moisture, pH, C availability, translocation of soil particles, and soil microbial biomass. We anticipated that the movement of organic matter throughout the soil matrix would lead to measurable changes in soil properties. Specifically, we tested the following two main hypotheses: (H1) *R. flavipes* will accelerate the transfer of organic materials from dead wood to the soil by feeding on dead wood, increasing available soil C and in turn the biomass of soil microbial communities. We therefore expected wood species that termites decomposed most rapidly to be associated with the greatest increases in available soil C and microbial biomass. (H2) *R. flavipes* will alter soil pH, moisture and mix soils through their tunneling and feeding activities. We expected termites to increase soil mixing, but it was unclear as to the direction of their influence on pH and moisture. We reasoned that they might acidify soil by promoting decomposition, or make it less acid by liberating elements such as calcium that are abundant in wood. Similarly, we reasoned that they might increase soil moisture by building soil organic matter, or decrease moisture by promoting drainage.

2. Materials and methods

2.1. Experimental design

Mineral soil was collected at 0–25 cm depth from East Rock Park forest, New Haven, Connecticut (41.32765° N and 72.90903° W). The soil was a fine sandy loam, mapped within the Rippowam series in the U.S. and following that classification a mesic Fluvaquentic Endoaquept. It was passed through a 4-mm sieve and stored at –20 °C for 48 h to defaunate the soil (Bruckner et al., 1995). To ensure that the freezing effectively killed all macro-invertebrates, the initial freezing period was followed by 24 h of thawing and a subsequent 24 h freeze. To track the movement of soil through the microcosm, the soil was then mixed with three unique colored sands. Specifically, waterproof red, blue and yellow sand (Activa Products, Inc., Marshall, TX, USA) was sieved to between 500 and 53 µm in diameter. The soil was homogenized and divided into three parts, and each part was mixed with a different color sand at a ratio of 20 parts soil: 1 part colored sand by dry mass. The now mixed soil was added to seventeen 28.6 × 32.1 × 31.8 cm plastic boxes (Cambro Manufacturing Company, Huntington Beach, CA, USA) in three, 6-cm deep layers – ordered yellow–blue–red from bottom to top.

Kiln-dried but otherwise untreated wood blocks were next added to each of the 17 soil microcosms. Each block measured

10 × 10 × 1.25 cm, and was sourced from red maple (*Acer rubrum*), northern red oak (*Quercus rubra*), eastern white pine (*Pinus strobus*) and yellow birch (*Betula alleghaniensis*) grown in Connecticut. The wood blocks were weighed and then soaked for 1 h in distilled water before addition to the microcosms, because moist wood is more palatable to termites. One wood block of each of the four species was placed on the soil surface and an additional four wood blocks were placed 3-cm below the surface (8 blocks total per microcosm). The four blocks in each layer were located in the corners of the box, about 1 cm from the edge and 10 cm apart, and each buried wood block was located beneath a wood block of the same tree species. Although we use a design where termites could “choose” wood, our intention was simply to ask how termite wood-feeding affected soil properties and whether these effects were wood-species dependent.

The microcosms were covered with black plastic bags on three sides and with a double-layered fine mesh at the open end, ensuring that light only came from above given that *R. flavipes* is negatively phototropic. Termite individuals were also collected in the New Haven area from two colonies and added to the center of 12 of the 17 microcosms four times over the course of the experiment to ensure termite populations were maintained (referred to as ‘termite-present’ microcosms). Each addition consisted of 200 individuals (800 total) with a soldier-to-worker ratio of 2:100. These numbers equate to field densities of 9000 individuals per m², and are comparable to the higher range estimate by Wood and Sands (1978) and Bignell and Eggleton (2000) for termites in natural ecosystems. Termites were not added to five remaining microcosms, which served as experimental controls (denoted ‘termite-absent’ microcosms). The microcosms were maintained at 20 °C in the laboratory and an equal amount of aged deionized water was sprayed every 3–4 days to prevent desiccation of the wood and surface soil.

2.2. Soil and wood analyses

The microcosms were systematically deconstructed after 25 weeks. The upper wood blocks were removed from the box and visible termites beneath and inside the wood were collected using an aspirator and stored at –20 °C for enumeration. Soil was then excavated below the wood block, from a 10 × 10 × 3 cm (depth) layer, exposing the corresponding buried wood block. This block was then removed from the soil and visible termites were collected in a separate vial. Soil cores were then excavated from beneath each buried block, but only to 2 cm more depth. The soils from above and below each buried wood block were mixed, hand sifted to remove termites, and stored at 5 °C for a few days to two weeks for all of the later analyses. The number of termites found during hand sifting was recorded. The wood blocks were then thoroughly cleaned using a dry hair brush, signs of termite infestation recorded, and the individual blocks placed in plastic bags and stored at 5 °C. The wood block was marked as infested if it showed any visible sign of termite infestation, regardless of presence or absence of termites in the block during the time of harvest. Lastly, within each microcosm, the top 5 cm of soil located at least 3 cm away from all the wood blocks was collected to serve as ‘away’ (from wood) soil.

Soil was analyzed for gravimetric soil moisture, water holding capacity, pH, active microbial biomass and microbially-available C following Bradford et al. (2008). Briefly, gravimetric soil moisture was determined by drying soil for 24 h at 105 °C. Water holding capacity was determined by saturating the soil with water in filter paper and finding the mass of water retained after 2 h. Soil pH was measured in a 1:1 water-to-soil volumetric ratio using a benchtop pH meter. Active microbial biomass was estimated through substrate-induced respiration (SIR), following a modified technique

(Fierer et al., 2003). Microbially-available C was estimated using a 30-d carbon mineralization assay, with the soil maintained at 85% water holding capacity instead of 65% given its sandy texture. The C mineralization assay provides an estimate of microbially-available carbon and, like all soil carbon fractionation techniques (physical, chemical and biological), its ecological relevance is operationally defined (Olk and Gregorich, 2006). Therefore, the measure is simply an index of the overall quality (the availability and the lability) of the C substrates that are being catabolized by decomposer organisms at a given point in time (Fierer et al., 2005). Carbon mineralization was measured five times, on days 1, 5, 10, 21 and 30. For each measurement 6 g of soil, in 50 mL centrifuge tubes fitted with gas-tight lids with septa for gas sampling, were flushed with CO₂-free air and incubated at 20 °C for 24 h. Headspace CO₂ concentrations were measured using infra-red gas analysis (Li-COR model LI-7000, Lincoln, NE, USA). Cumulative C mineralized was determined by integrating CO₂-efflux rate values across all measurements.

The wood blocks were weighed and drilled in a regular array using an 8.5-mm drilling bit to extract sawdust. The dust was mixed thoroughly and was dried at 65 °C to constant mass. The drilled wood blocks were weighed again, and dried at 85 °C to constant mass. The dried wood blocks were reweighed to determine moisture content and dry wood biomass. The process of drilling, weighing and drying was also carried out for 12 additional wood blocks (3 of each species), not incubated in the microcosms, to calculate the average initial concentration of C and nitrogen (N) in wood in each species. Sawdust was ball-milled to a fine powder and analyzed for C and N concentration using an ECS 4010 Elemental Analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA). Total mass of C and N in wood was calculated by multiplying dry mass of wood with C and N concentration in wood, respectively. Change in total mass of C and N in wood blocks was then determined by subtracting the final mass of C and N in wood blocks from initial mass of C and N in wood blocks, respectively.

2.3. Movement of soil particles

For estimation of vertical movement of soil particles by termites, the proportion of red, blue and yellow sand within the surface soil stratum (i.e. top 5 cm, red sand) was estimated. Soil (5 g) was washed with water between two sieves: 500 µm on top and 53 µm on the bottom to retain sand-size particles. An aliquot of the particles retained in the bottom sieve was dispersed with water as a thin smear on a finely graduated (1 × 1 cm) Petri dish. Starting from a fixed cell in the dish, all three color particles (yellow, blue and red) were counted under a dissecting microscope. Counting continued until the number of red sand particles exceeded 500, at which point counting of the remaining particles in the current cell was completed and no new cell was started. The proportion of blue or yellow sand particles to red in the sample was used as an indicator of the extent of soil mixing.

2.4. Statistical analyses

Linear mixed-effect models were used to investigate the effects of termites, wood species, and wood position on soil and wood variables. The mixed-effect modeling framework accounted for auto-correlation, as measurements in the same microcosm were spatially non-independent. Two sets of models were constructed: one investigating the effects of termite activity on soil properties, and one investigating their effects on wood decomposition. Fixed effects in the soil models were termites (presence or absence) and proximity to wood species (pine, maple, oak, birch, or away). Fixed effects in the wood models were termite (presence or absence), wood species and position (surface or buried). Microcosm number

was used as the random intercept for both sets of models. We selected minimally adequate models using lowest AIC. Variance inflation factors of <2 indicated that collinearity was low between model variables. For retained fixed effects and their interaction, we considered $p < 0.05$ significant and $p < 0.10$ marginally significant (Hurlbert and Lombardi, 2009). We checked the models to ensure there was no heteroscedasticity in the residuals and that the response variables were a reasonably linear function of the fitted values, with errors closely distributed. A chi-square test of association was conducted to test the relationship between wood type and likelihood of infestation by termites and wood position. All analyses were conducted using the freeware statistical package R (<http://cran.r-project.org/>).

3. Results

3.1. Soil properties

Termites consistently increased microbially-available C, as did wood presence (Fig. 1a; termite: $F_{1,15} = 10.6$, $p = 0.005$; wood: $F_{4,64} = 19.1$, $p < 0.001$; see Tables S1–S8 for model coefficients for the best fit models of all of the response variables). Termite presence was associated on average with microbially-available C values of 145 ± 3.9 and absence with values of 121 ± 3.2 µg CO₂ g dry wt. soil⁻¹ 30 d⁻¹, giving ~20% higher available C when termites were present. Microbial biomass was consistently higher on average with termites (Fig. 1b), although this effect was not significant ($F_{1,15} = 1.24$, $p = 0.283$). In contrast, microbial biomass was significantly higher in soil underneath as opposed to 'away' from wood blocks ($F_{4,64} = 19.8$, $p < 0.001$).

Consistent termite effects, independent of the wood species, were also mirrored in the other soil properties. That is, there were no significant interactions between termites and wood for any of the measured soil properties, although marked effects of each main effect was observed. Specifically, the best-fit model for soil pH retained wood species ($F_{4,64} = 67.2$, $p < 0.001$) and termites ($F_{1,15} = 5.27$, $p = 0.037$), where the presence of wood generally made the soil less acidic and termite presence consistently increased soil acidity (Fig. 1c). On average, termite presence resulted in a soil pH value of 4.72 ± 0.03 and absence resulted in a value of 4.84 ± 0.04 , representing ~32% higher acidity in the presence of termites.

The best fit model for soil moisture also retained termites ($F_{1,15} = 7.58$, $p = 0.015$) and wood ($F_{4,60} = 5.77$, $p = 0.005$). There was a marginally significant wood by termite interaction ($F_{4,60} = 2.19$, $p = 0.081$), although it was unclear as to why this interaction occurred because the termite and wood species effects appeared consistent (Fig. 1d). Specifically, soil moisture was consistently lower with termites, where on average moisture was 18.6% without termites and 15.5% with termites, a relative decline of ~20%.

3.2. Wood decomposition

The percentage of wood blocks infested by termites ranged from 67% for the buried maple and birch blocks, to 100% for the surface maple blocks (Table 1). No significant relationship between wood species and the likelihood of termite infestation was observed ($\chi^2 = 1.37$, $df = 3$, $p = 0.713$). There was also no significant relationship between vertical placement (surface versus buried) of wood blocks and likelihood of infestation ($\chi^2 = 2.46$, $df = 1$, $p = 0.117$). Mass loss was observed in all wood species for both surface and buried positions except for the surface pine in the termite-absent microcosms (Fig. 2a), albeit marked increases in the total mass of N suggested that the wood biomass was being

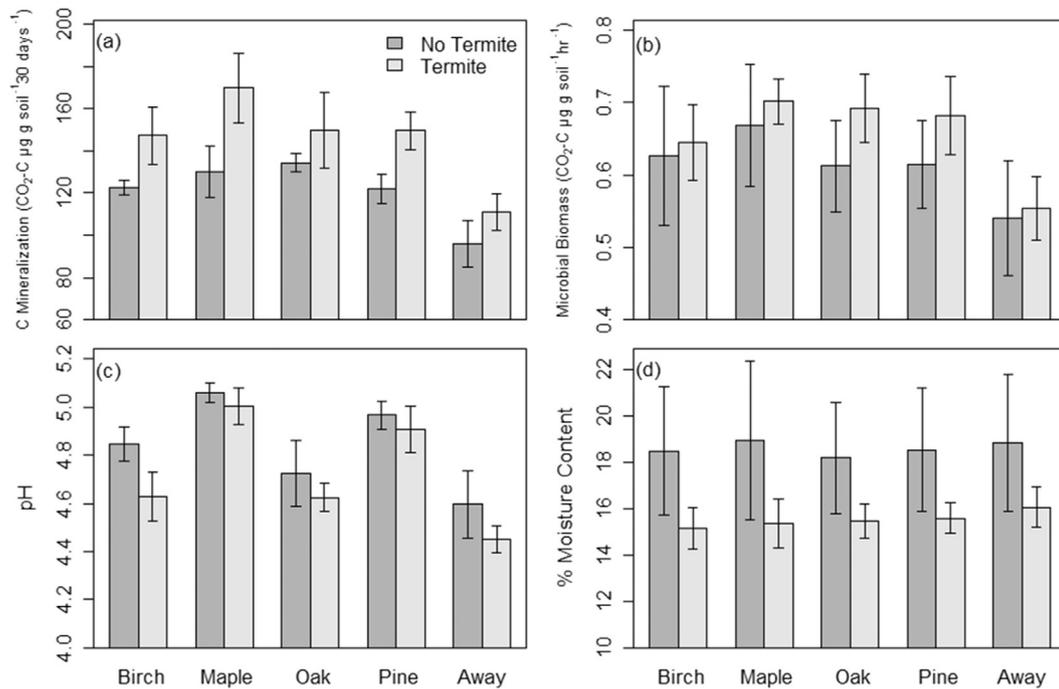


Fig. 1. Effect of wood and termites on soil properties: (a) microbially-available carbon, (b) microbial biomass, (c) pH (following back transformation from H^+ ion concentration), and (d) gravimetric moisture. Values are means with SE bars; $n = 12$ for termites present and $n = 5$ for termites absent.

transformed through decomposition processes (Fig. 2c). Visible symptoms of termite infestation (burrows) were observed in the majority of wood blocks (but not in termite-absent microcosms). Average mass loss in termite-present wood ($3.48\% \pm 0.30$) was 77.5% higher than in termite-absent wood ($1.96\% \pm 0.47$). The best-fit model for wood mass loss retained termites ($F_{1,15} = 13.2$, $p = 0.003$), wood species ($F_{3,111} = 59.2$, $p < 0.001$) and wood position ($F_{1,111} = 58.9$, $p < 0.001$). The interactions between wood species and termite ($F_{3,111} = 4.30$, $p = 0.007$), and termite and wood position ($F_{1,111} = 4.65$, $p = 0.033$), were also significant indicating that effects of termites on wood mass loss were dependent on both wood species and position (Fig. 2a).

Net C mass loss occurred in wood blocks of all species except for the blocks that were buried in the termite-present microcosms (Fig. 2b). The best-fit model for C mass remaining retained termites ($F_{1,15} = 16.3$, $p = 0.001$), wood species ($F_{3,114} = 13.4$, $p < 0.001$) and position ($F_{1,114} = 246$, $p < 0.001$). The only significant interaction was between termite and position ($F_{1,114} = 87.2$, $p < 0.001$), highlighting that termite effects were dependent on position, likely because of the gain in mass of C in the buried blocks when termites were present (Fig. 2b). Net gain in mass of N was observed for all species except for buried birch without termites (Fig. 2c). The best-

fit model for N mass remaining retained termites ($F_{1,15} = 6.88$, $p = 0.019$), wood species ($F_{3,112} = 66.1$, $p < 0.001$) and the interaction between wood species and position ($F_{3,112} = 6.35$, $p < 0.001$). The interaction indicated that decomposition of wood species was depth dependent, as well as being influenced by termite presence.

3.3. Soil engineering

Construction of vertical mud tubes on the soil and wood surfaces (pers. obs. by the authors) indicated that *R. flavipes* are capable of transporting soil particles upward. However, a negligible number of blue or yellow colored sand particles were found in the upper-most soil layer. The average number of blue sand particles in termite-present soil was 1.3 ± 0.2 and that for termite free soil was 0.7 ± 0.2 per 1000 red particles, a difference that was not statistically significant (termite effect: $F_{1,15} = 2.42$, $p = 0.14$).

4. Discussion

Field studies illustrate a role for temperate termites in governing wood decomposition rates (Gentry and Whitford, 1982; Warren and Bradford, 2011; Bradford et al., 2014), yet their impacts on soil

Table 1

Wood blocks with termite infestation. In just the wood blocks and local excavated soils, 29.8% on average of the total number of added termites was recovered at the experiment end.

Wood species and position	Wood blocks showing signs of infestation (% total blocks; $n = 12$)	Wood blocks with termites present at the experiment end (% total blocks; $n = 12$)	Number of termites retrieved in the wood blocks and local soil (mean \pm SE; $n = 12$)
Pine surface	92	58	29 ± 13
Pine buried	83	58	11 ± 5
Oak surface	75	38	60 ± 22
Oak buried	83	42	12 ± 8
Maple surface	100	63	57 ± 13
Maple buried	67	33	15 ± 5
Birch surface	83	67	30 ± 7
Birch buried	67	58	25 ± 13

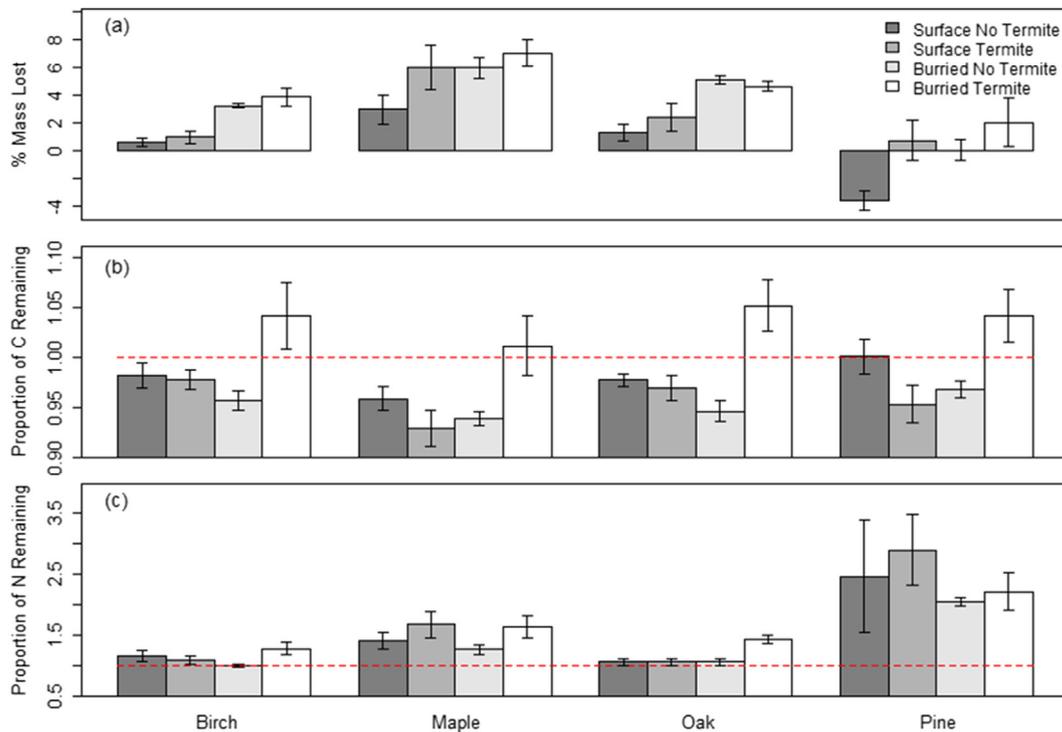


Fig. 2. Wood mass loss, carbon and nitrogen dynamics as influenced by wood species, termites and whether buried or surface wood. (a) Percentage mass loss from wood; (b) proportion of initial carbon remaining in the wood block, where the red dashed line represents initial wood carbon mass; (c) proportion of initial nitrogen remaining in the wood block, where the red dashed line represents initial wood nitrogen mass. Values are means with SE bars; $n = 12$ for termites present and $n = 5$ for termites absent. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

properties are largely unknown. We show that temperate termites have strong and consistent (across wood species) effects on microbially-available soil C, soil moisture, and pH, as well as positive but non-significant effects on microbial biomass. Although we hypothesized effects on these soil properties, we expected that the magnitude of effect on available C and microbial biomass would correlate positively with the rate at which different wood species decomposed (H1). However, the absence of significant interactions between termites and wood species highlighted that these species-dependent effects did not occur, corroborating the consistent mean responses (Fig. 1). Our data therefore suggest that it is the activities of termites in the soil matrix, as opposed to their feeding rates per se, that drive soil responses.

We were less certain about the direction of termite effects on soil pH and moisture (H2), but our observations highlight a potential role for termites in acidifying soils and reducing soil moisture. We were surprised that termites had only a negligible effect on upward movement of soil given their anticipated engineering effects (Black and Okwakol, 1997; Dangerfield et al., 1998; Jouquet et al., 2011), yet their influence on the other measured soil properties suggest that they could still have strong effects on forest biogeochemical cycling and species composition.

Termites activities presumably contributed organic material to the soil – due to the deposition of woody debris, termite byproducts, and termite necromass – resulting in increased soil available C (Zaady et al., 2003; Ackerman et al., 2007). Incorporation of feces and saliva from termites is known to affect soil microorganisms (Jouquet et al., 2011), generating higher microbial abundances, activities and diversity in their nests compared to surrounding soils (Gupta et al., 1981; Black and Okwakol, 1997; Holt and Lepage, 2000; Chouvenec et al., 2011). In addition, termites regulate microclimatic conditions inside their nest (Blackwell and Rossi, 1986) and

contribute to the formation of soil microaggregates (Lavelle et al., 1997; Jungerius et al., 1999), both of which enhance microbial growth. Our data suggest that the temperate termite, *R. flavipes*, despite its lack of mound building, also has the potential to modify the soil environment in a manner that promotes microbial activity and biomass (Pitts-Singer and Forschler, 2000). Hence, the known positive effects of the decomposition of woody debris on dissolved organic C supply to soil (Yavitt and Fahey, 1985; Spears et al., 2003; Kahl et al., 2012), stimulating decomposer microorganisms (Graham et al., 1994; Kappes et al., 2007; Marañón-Jiménez et al., 2011), seems to be amplified by temperate termites.

Lower soil pH due to termite presence is consistent with previous observations that logs channelized by macroinvertebrates have lower pH during the early stage of decomposition (Kitchell et al., 1979). However, other studies have shown idiosyncratic effect of termites on pH, depending on the termite species and soil type. For example, Donovan et al. (2001) reported that the soil-feeding termite, *Cubitermes fungifaber*, raised soil pH in two sites, lowered it in one site and had no effect in two sites. Nutting et al. (1987) found that subterranean termites in Sonoran Desert grassland, *Heterotermes aureus* and *Gnathamitermes perplexus*, slightly increased the soil pH by bringing clay-rich soil from depth to the surface. We did not observe significant soil movement and indeed started with homogenous soils. Nevertheless, *R. flavipes* increased acidity, whereas the presence (versus absence) of wood decreased acidity (Fig. 1) as is commonly observed (Müller et al., 2005; Topp et al., 2006; Kappes et al., 2007). The most parsimonious explanation for the termite effects on soil pH then seems to be increased decomposition activity in the soil (which generates protons) due to increased organic matter from wood decomposition and termite secretions, but further work is required to elucidate the specific mechanism(s).

We were able to recover ~30% of the termites from the wood blocks and the soil local to them that we then used for the soil analyses. These termite abundance assessments were intended to provide a relative comparison of the proportion (of those added) that were active at harvest in the zone of the wood. If we instead use these recoveries as an estimate of the maximum potential mortality in our microcosms, we get a value < 70%. Such mortality rates are approximately equivalent, or substantially lower, than previous reports (Smythe et al., 1971; Harahap et al., 2005). McManamy et al. (2008) estimated that the average lifespan of *R. flavipes* collected under field conditions is ~180 days, and possibly up to 1 year. A mortality rate of ~70% over 25 wks (175 d) is then in line with such estimates. As such, there is the possibility then that some of the changes in soil properties that we observed resulted from termite necromass serving as a substrate for decomposition, but such inputs could be considered a natural process in termite colonized soil (as opposed to an artifact of our experimental design).

Tunneling by termites creates channels in soil through which water preferentially flows (Léonard and Rajot, 2001; Léonard et al., 2004; Turner, 2006). Termite foraging and hence tunnel building therefore likely increased water infiltration rates, resulting in the lower moisture in the termite-present microcosms (Fig. 1d). When temperatures are above 10 °C, most of the biomass of *R. flavipes* in temperate forests is likely found in dead and decaying wood (King et al., 2013). Hence we might expect soil drainage and aeration characteristics to differ markedly between areas with high versus low densities of woody debris when termites are present.

The extent to which temperate forest termites vertically move soil particles is unresolved (Maynard et al., 2015). We found upward translocation of soil from below 6-cm depth, albeit the amount brought up was very minimal, and we did not quantify whether termites moved soil horizontally or downward. Some studies show that subterranean termites, including *Reticulitermes hesperus* (Ebeling and Pence, 1957), *Coptotermes brunneus* (Greaves, 1962), and *C. acinaciformis* (Greaves and Florence, 1966), form tunnel space by compacting soil rather than transporting the particles. Further, although Li and Su (2008) reported that *C. formosanus* did transfer soil, they found that transfers were minimal, where they instead constructed their extensive subterranean galleries using space gained during wood consumption. In contrast, Nutting et al. (1987) estimated that subterranean termites in arid grassland, *Heterotermes aureus*, *Gnathamitermes perplexus* and *G. tubiformans*, moved substantial amounts of soil (Nutting et al., 1987). Our data and other observations for temperate forests therefore suggest that termites are not major agents of soil movement, but downward and horizontal soil transfers should be investigated.

Termite presence increased wood decomposition rates by 77% on average and termites exhibited no clear preference for any one wood species. The gain in mass in surface pine blocks in the absence of termites was unexpected and could be due to fungal biomass growth in the wood facilitated by translocation of nutrients from the surrounding soil (Grier, 1978; Graham and Cromack, 1982). Accumulation of fungal biomass in wood blocks was evident on visual examination both during the incubation and the harvest time and, although the change in mass of pine wood blocks appeared idiosyncratic, the large and consistent gains in total mass of N showed that substantive chemical transformations of the wood were occurring with accumulation of N from exogenous source that are expected with its decomposition (Sinsabaugh et al., 1993). Loss of C mass from wood blocks followed the same trend as mass loss (Fig 2), except for the buried wood with termites, which instead gained C mass and lost net total mass. It is unclear as to what might drive these gains in mass of C in wood, and future work

should assess whether C-rich termite secretions might explain the pattern we observed.

Termites significantly increased mass of wood N for all species, particularly in the surface wood blocks (Fig. 2). Total mass of N in wood increased in all wood blocks, regardless of termite presence/absence, except for the surface birch blocks in the non-termite microcosms. These results are consistent with observations that wood N concentrations often increase as decomposition progresses (Laiho and Prescott, 2004; Garrett et al., 2008; Lyons et al., 2010). The increase in N mass in the wood blocks could be due to transfer of soil N into the wood by wood-rot fungi (Whitford, 2002) and/or N fixation by bacteria syntrophically associated with the fungi (Grier, 1978; Graham and Cromack, 1982).

Although termites exhibited no clear preference for any one wood species, it was surprising that the different decomposition dynamics across the wood species did not translate to wood-species dependent effects on the soil properties. Admittedly, our experiment was not designed as a true choice experiment and it is possible that termites moved between wood blocks (Long and Thorne, 2006), potentially removing wood-species dependent, as well as “away from wood”, effects on the soils. If the different wood decomposition dynamics are indicative of termite preferences and are transmitted to soils, then there is the potential that under field conditions termite “choice” could be an important arbiter of termite effects on soils. Under such conditions, the relative and absolute abundance of different wood species might then regulate termite effects on soils.

An important question is how our experimental design influences the validity of extrapolating our microcosm results to how termites influence soil properties and ecosystem processes under field conditions. Our results perhaps then should only be considered to show underlying mechanisms and the potential qualitative effects (increase, decrease, no change) of termites on soil properties. For example field soils receive other organic inputs, such as those from fine root turnover and exudation, which drive variation in soil properties in space and time. Field studies are required to discern the biological significance of our findings for the ecology of temperate forests in light of other inputs. In addition, we had no primary reproducers in our experiment and their absence, in microcosm studies (Bulmer and Traniello, 2002), has been shown to alter termite behavior and it is unknown as to how their absence might affect termite effects on ecosystem processes. Yet the magnitude of the effects we observed – for example a 20% difference in microbially-available C and moisture availabilities, shows the potential that termites might have strong effects on soil biogeochemistry and plant community composition. These effects occurred over and above control (i.e. no termite) soils, where the wood blocks also contributed organic matter to the soil as evidenced by the increase in microbially-available C, suggesting that termites augment dead wood effects on soil properties.

Despite necessary cautions about extrapolating microcosm results to field dynamics, our results show that temperate termites have the potential to play an important role in shaping surface soil properties. Termite-induced changes in soil moisture, C availability, pH and microbial biomass may have subsequent effects on soil C storage, plant community composition, nutrient and water cycling. Notably little work has been done on this cryptic but often highly abundant guild of temperate forest-dwelling insects, confounding the ability to estimate with any certainty their current and future impacts on temperate forest structure and functioning. Such estimates are especially important because temperate termites are projected to move poleward and become locally more abundant at higher latitudes as the climate changes.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.soilbio.2015.08.025>.

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