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Elemental concentrations in the frass of saproxylic insects suggest a role in micronutrient cycling

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Abstract. Concentrations of 22 elements in pinewood were compared with that in frass produced by insects representing the following taxa: *Reticulitermes* spp. (Rhinotermitidae), *Zootermopsis nevadensis* (Termopsidae), *Incisitermes snyderi* (Kalotermitidae), *Hylotrupes* spp. (Cerambycidae), *Heterobostrychus* spp. (Bostrichidae), *Lyctus* spp. (Bostrichidae), and representatives of the family Ptinidae (formerly Anobiidae). Twenty elements (Al, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, Si, Sr, and Zn) were measured using inductively coupled plasma-optical emission spectroscopy (ICP-OES), whereas carbon, hydrogen, and nitrogen percentages were measured using a CHN autoanalyzer. Chromium was the only element present at a statistically lower concentration in all frass types compared to pinewood. A comparison of pinewood to frass from those taxa that fed on pine revealed that *Reticulitermes* frass contained significantly higher levels of 15 elements, *Zootermopsis* 10, Ptinidae 5, *Incisitermes* 4, and *Hylotrupes* 1. Only *Incisitermes* frass showed a significantly higher percent carbon than pinewood and *Reticulitermes*, *Zootermopsis*, and Ptinidae showed significantly higher percent nitrogen. Examination of percent approximate digestibility (PAD) indicated that *Reticulitermes* frass had 14 elements that were ≥200% more concentrated than found in pinewood, whereas *Zootermopsis* had 6, *Lyctus* 5, ptinid 4, *Hylotrupes* and *Heterobostrychus* 3, and *Incisitermes* none. This survey of elements in frass indicates that saproxylic insects are, for the most part, not sequestrating but rather recycling (releasing) the store of micronutrients in wood biomass, with the greatest potential contribution to soil nutrient cycles attributable to subterranean termites.

Key words: feces; frass; southeastern USA; termites; trace metals; trace minerals; wood-feeding insects.

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Introduction

Arthropods are recognized as ecosystem engineers in a number of habitats, including temperate forests (Jones et al. 1994, Lavelle et al. 2006, Jouquet et al. 2011). Ecological studies aimed at determining the effects of arthropods on nutrient cycling in forest ecosystems have been centered on the employment of mesh litterbags (Liu et al. 2001, Ball et al. 2009, Carrillo et al. 2011, Ashton et al. 2012), useful mainly for examining seasonal nutrient releases from senescent leaves. Perhaps the reasoning on such extensive litterbag research is that foliage contains the highest fraction of microelements compared to other tissues (Young and Guinn 1966, Whittaker et al. 1979, Arthur and Fahey 1992, Hagen-Thorn and Stjernquist 2005, Saarela et al. 2005). Other studies have shown that canopy herbivore frass plays a role in nutrient cycles by returning plant organic matter to soil nutrient reserves (Hollinger 1986, Hunter et al. 2003, Fonte and Schowalter 2005, Schowalter et al. 2011, Kagata and Ohgushi 2012). However, Whittaker et al. (1979) determined that the nutrient pools of C, N, P, S, Ca, K, Mg, Mn, Na, Fe, Zn, and Cu in woody tissues of standing trees exceed those in leaves and the concentrations of most elements were similar in living and dead wood. Therefore, studies that neglect the fibrous structural tissue found in the stems, trunks, and roots of woody plants underrepresent the majority of total forest plant biomass (Arthur and Fahey 1992, Xiao et al. 2003).

There is general agreement that standing dead trees and coarse woody debris (CWD) are important structural components of the forest environment that provide habitation for arthropod populations important in the comminution and mineralization of organic debris (Jabin et al. 2004), however, the literature on dead wood and nutrient cycling provides contradictory information. While some authors claim that CWD plays a negligible role in nutrient dynamics in comparison to litter (Laiho and Prescott 2004, Kim et al. 2006), Abbott and Crossley (1982) concluded that leaving CWD on forest floors aids in nutrient conservation. The decomposition of CWD may release more nutrients over a longer temporal scale than the seasonal flush of nutrients provided by the decomposition of leaf litter.

The influence of saproxylic insect activity on nutrient dynamics is rarely acknowledged. Saproxylic arthropods open infection courts conducive for the proliferation of other organisms, furthering the nutrient leaching process through channelization and fragmentation; however, little quantitative data are available to elucidate the extent of this role (Hanula 1996, Ulyshen and Wagner 2013). Wood-feeding insect populations represent a large portion of forest soil living biomass, with subterranean termites comprising approximately 45% of the overall soil macrofaunal biomass in eastern USA deciduous forests (King et al. 2013). Despite their abundance, little research has been conducted on the frass of termites or wood-feeding beetles. Brune (2014) reviewed the efficiency at which termites digest lignocellulose but did not mention the nutritional content of the frass or the potential role of termites in micronutrient recycling. Geib et al. (2008) found significant levels of propyl side-chain oxidation (depolymerization) and demethylation of ring methoxyl groups in lignin after passing through the alimentary canal of two insect species, *Anoplophora glabripennis* (the asian longhorned beetle) and *Zootermopsis angusticollis* (the Pacific dampwood termite). Similarly, Ke et al. (2011) examined the feces of *Coptotermes formosanus* to record how lignin was modified, but not the nutrient content. In general, saproxylic insects represent a significant portion of the forest arthropod community that modify the physical and chemical properties of coarse woody debris. Further research will be needed to address if these modifications result in the egestion of excess nutrients into the environment or the sequestration of limiting nutrients from wood.

There are a number of studies that report elemental concentrations in the alimentary tract of selected saproxylic insects. Vu et al. (2004) examined the hindgut contents and fluid of *Zootermopsis nevadensis* (a dampwood termite) and *Incisitermes minor* (a drywood termite) and found varying concentrations of K, Mg, Ca, Fe, Zn, Al, Ba, Cu, and Mn. Potassium was consistently present at the highest concentrations (3000 ppm or greater) in the hindgut contents of these termites when compared to the remaining elements, which were present in concentrations between 5 and 440 ppm (Vu et al. 2004). Both Yoshimura et al. (2002) and Stewart et al. (2011) observed higher concentrations of metals, especially Mg, Al, P, Ca, Zn, in the gut of termites compared to other body parts. Esenin and Ma (2000) concluded that concentrations of Zn, Cu, and Cd in cerambycid larval frass were similar to concentrations in the phloem or xylem where they fed. Cobb et al. (2010) found that the frass of a pyrophilous cerambycid beetle, *Monochamus scutellatus*, altered nitrogen availability in boreal forests recovering from wildfire. Based on the available evidence, saproxylic insect frass may play a larger-than-expected role in micronutrient cycling in forest ecosystems.

Although the mineral content of saproxylic insect frass has not generated much research, the elemental content and biomass in temperate forest stands has been documented. Wood comprises the majority of plant biomass in forests but a large disparity exists in the concentrations of microelements present in wood, with elemental concentrations varying between tissues within a species (Young and Guinn 1966, Whittaker et al. 1979, Hagen-Thorn and Stjernquist 2005), and between species (Young and Guinn 1966). Therefore, the mineral content returned to the soil by saproxylic insect activity remains unresolved.

In this study, the chemical composition of frass produced by several genera of saproxylic insects was analyzed in order to determine if trace elements locked in the cellulose lattices of CWD are being excreted in their frass. The objective of this study was to determine and compare the concentration of 22 elements (Al, B, Ba, C, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, N, Na, Ni, P, Pb, Si, Sr, and Zn) in pinewood and frass from *Reticulitermes* spp., *Zootermopsis nevadensis*, *Incisitermes snyderi*, *Hylotrupes* spp., *Heterobostrychus* spp., *Lyctus* spp., and representatives of the family Ptinidae. Frass types were selected based on availability of insect laboratory cultures or infested structural lumber. We hypothesized that the concentrations of elements in saproxylic insect frass would be no different than those in wood.

Methods

The frass from saproxylic insects representing six genera (*Reticulitermes* spp., *Zootermopsis* spp., *Incisitermes* spp. *Heterobostrychus* spp., *Lyctus* spp., and *Hylotrupes* spp.) as well as the family Ptinidae (genera or genus unknown) from two orders (Blattodea and Coleoptera) was collected from field sites or laboratory cultures, the latter maintained with pinewood alone as a food resource. Samples are randomly selected portion(s) from a specified source of frass or pinewood. We defined source as a location where samples were obtained. Appendix S1 lists the source for each frass and pinewood sample, with multiple samples taken from certain sources to account for the potential variability. All termite frass samples were obtained from the University of Georgia Household and Structural Entomology Laboratory cultures, except for two *Incisitermes* samples collected from field sites. There were six sources of *Incisitermes* frass and seven sources of *Reticulitermes* and *Zootermopsis* frass. All pinewood samples except one were dimensional lumber, a term used to describe timber that is finished/planed and cut to standardized dimensions (Appendix S1).

Heterobostrychus frass was identified based on adult specimens collected from infested wood. Ptinid, *Lyctus*, and *Hylotrupes* frass were determined on frass texture, emergence hole diameter and shape because the insects were not found *in situ* (Ibach 2013). Ptinid and *Heterobostrychus* beetles are known to feed on both hardwood and softwood. All Ptinid frass samples used in this study originated from structural pine lumber (Appendix S1) and were therefore categorized with *Hylotrupes* and the termites as pine-feeders. One of our two *Heterobostrychus* frass sources originated from hardwood (Appendix S1), whereas all the *Lyctus* frass sources were from hardwood. Therefore, the differences in element concentrations in our *Heterostrychus* and *Lyctus* frass samples, compared with pinewood, may be attributed to disparities between coniferous and deciduous species.

Reticulitermes and *Zootermopsis* frass were collected from laboratory cultures in which termites were kept in plastic boxes with only wood. The organic debris deposited onto the surface of the culture boxes were collected as frass. *Incisitermes* frass was identified and collected based on the characteristic shape of the fecal pellets. Field collected *Incisitermes* fecal pellets were collected on site and stored in glass vials until sample preparation. All cultured termite frass samples were collected and placed in 16.51 × 17.46 cm Press-N-Seal plastic bags and air-dried at room temperature for approximately 1 week prior to sample preparation. Samples were examined under a dissecting microscope to remove extraneous material such as fibrous wood particles and miscellaneous insect parts. Samples were crushed to a fine powder with mortar and pestle, weighed, placed in 7.62 × 10.16 cm Press-N-Seal plastic bags, and labeled. The forceps, mortar, and pestle were thoroughly scrubbed with detergent, rinsed, and dried using a paper towel between sample preparations. Wood samples were ground to a fine powder using a Wiley mill and analytical ball mill, provided by the Pete Philips Laboratory for Nutrient Cycling Science at the University of Georgia, weighed, placed in 7.62 × 10.16 cm Press-N-Seal plastic bags, and labeled.

All chemical analyses were conducted at the Chemical Analysis Laboratory, University of Georgia Center for Applied Isotope Studies. Percent carbon and nitrogen were determined using a CHN analyzer and the concentrations (mg/kg) of the following 20 mineral elements: Al, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na,

Table 1. Percent approximate digestibility (PAD) values for median element concentrations measured by ICP-OES.

Element	Frass types						
	r	z	i	y	p	e	l
Al	**−935**	**−1081**	25	74	−338	26	**75**
B	−287	−43	−22	−18	**−1409**	−32	−131
Ba	**−885**	**−322**	−101	−44	−75	31	**−574**
Ca	**−584**	**−130**	**−139**	−8	**−55**	−30	−126
Cd	**−50**	0	0	0	0	0	**−11**
Co	0	0	0	0	0	0	0
Cr	**68**	**89**	**86**	**88**	**90**	**90**	**90**
Cu	**−315**	−33	**−35**	20	−31	−56	−83
Fe	**−205**	−268	**81**	88	38	**75**	**87**
K	**−258**	−55	−18	**−181**	−58	−309	**−375**
Mg	**−620**	**−231**	**−169**	−44	**−68**	**−57**	**−399**
Mn	**−449**	**−182**	−139	10	**−162**	−18	**73**
Mo	**−245**	**−146**	0	−26	−4	0	0
Na	−216	−10	−182	−291	**−1053**	−290	−72
Ni	9	49	49	49	49	49	26
P	**−350**	−87	**−152**	−384	−91	**−545**	**−554**
Pb	**−402**	**−257**	1	43	−290	43	−66
Si	**−674**	**−435**	23	82	−97	42	19
Sr	**−573**	**−169**	**−160**	−31	**−104**	−46	**−348**
Zn	**−479**	−184	−98	−219	−106	−157	6

Notes: PAD = $([E]_{wood}-[E]_{frass})/[E]_{wood} \times 100$; $[E]_{wood}$ represents the median element concentration of wood; $[E]_{frass}$ represents the median element concentration in a frass type. Statistically significant pairwise comparisons between frass and wood (shown in Figs. 1–4) are listed in bold. Abbreviations are as in Fig. 1.

Ni, P, Pb, Si, Sr, and Zn; were determined using ICP-OES. The ICP-OES and CHN raw data were organized by element for each frass type and pinewood, with concentrations below the detection limit adjusted to a value of 1 mg/kg. Residual histograms and boxplots were used to assess normality and homogeneity of variance. None of the data fit a normal distribution and all displayed heterogeneity of variance. Therefore, we used multiple Kruskal–Wallis (nonparametric ANOVA), followed by multiple Wilcoxon Mann–Whitney tests (nonparametric, two-sample *t*-test) to determine significantly different pairwise comparisons of frass types and pinewood, as well as between frass types. The significance level for all tests was $P < 0.01$. Median element concentrations were calculated for each category (frass types and wood). The nonparametric analyses were performed using the NPAR1WAY procedure, and medians calculated using the MEANS procedure in SAS version 9.3.

Approximated digestibility (AD) have been used to determine the fraction of consumed dry wood mass in insect feces (Mattson 1980, Slansky 1985, Grace and Yamamoto 2009). This calculation was modified to determine the percentages of element concentrations in wood that were egested as frass by using the following formula: AD = $([E]_{wood}-[E]_{frass})/[E]_{wood}$, where $[E]_{wood}$ is the median concentration in wood and $[E]_{frass}$ is the median concentration in frass (Table 1). Calculated AD values were rounded to the nearest whole number and reported as percent approximate digestibility (PAD). Our PAD values are synonymous to AD values or utilization efficiencies.

Results

The plant macroelements C, Ca, K, Mg, N, and P (Maathuis 2009) were present in two or more pine-feeder frass types at concentrations significantly higher than wood (Fig. 1). Median %C ranged from 46% to 53%, and represented the most abundant element found in all our frass and wood samples (Fig. 1). *Incisitermes* frass (53%) was the only frass type that contained higher %C than pinewood (47.8%) and all other frass

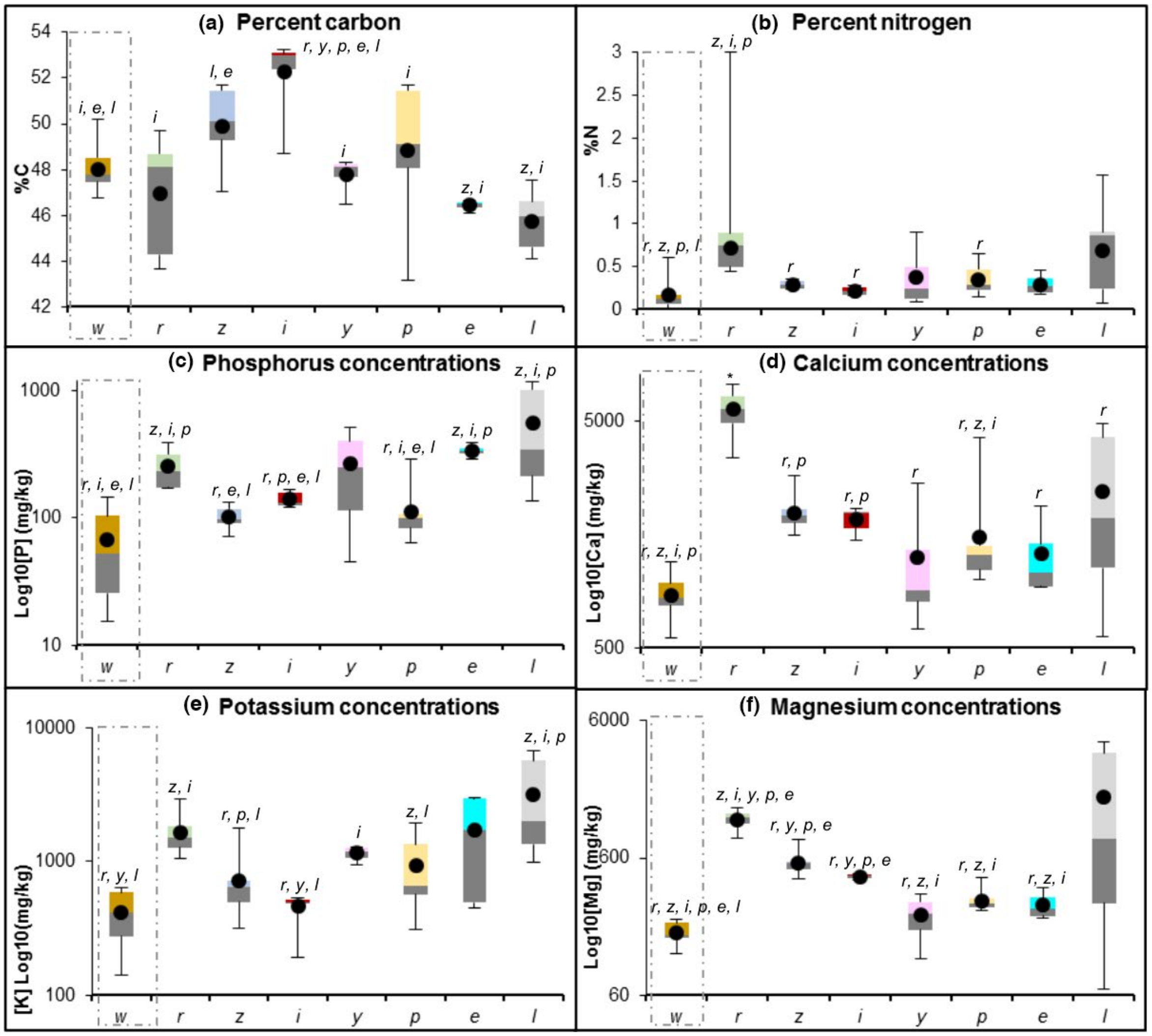

Fig. 1. Box and whisker charts showing concentrations of major macroelements (a–c) and plant essential macroelements (d–f) in pinewood and frass types. Element concentrations below the detection limit were adjusted to 1 mg/kg. The boxes are bound at the top by Q3 (third quartile) and at the bottom by Q1 (first quartile); medians divide each box and black dots represent means. The whisker bars extend from Q1 to the minimums, and from Q3 to the maximums. Letters above each box and whisker bar indicate significant differences (*P*-value = 0.01) between the element concentrations shown in the bar and the element concentrations of the categories listed along the *x*-axis. Abbreviations for the categories are: pinewood (*w*); followed by *Reticulitermes* (*r*), *Zootermopsis* (*z*), *Incistermes* (*i*), *Hylotrupes* (*y*), Ptinid (*p*), *Heterobostrychus* (*e*), and *Lyctus* (*l*) frass. An asterisk denotes a significant difference with all other frass types.

types, except *Zootermopsis* (Fig. 1). Median %N (ranging from 0.128% to 0.863%) was greater in *Reticulitermes*, *Zootermopsis*, and ptinid frass than pinewood, and was significantly higher in *Reticulitermes* frass than *Zootermopsis*, *Incisistermes*, and ptinid frass (Fig. 1). Phosphorus concentrations in *Reticulitermes* and *Incisitermes* frass were greater than concentrations in wood. *Reticulitermes*, *Heterobostrychus*, and *Lyctus* frass provided higher P concentrations than *Zootermopsis*, *Incisitermes*, and ptinid frass (Fig. 1). All termite and ptinid frass contained higher concentrations of Ca and Mg than wood (Fig. 1). *Reticulitermes* frass had higher Ca concentrations than all other frass types, and higher Mg concentrations than all other frass types except *Lyctus* frass. *Reticulitermes, Hylotrupes,* and *Lyctus* frass contained greater K concentrations than wood (Fig. 1). *Lyctus* frass provided the highest K concentrations and was significantly higher than *Zootermopsis*, *Incisistermes*, and Ptinid frass (Fig. 1).

All essential plant microelements (B, Cu, Fe, Mn, Mo, Ni, and Zn) (Hänsch and Mendel 2009) were present in significantly higher concentrations in at least one frass type than pinewood (Fig. 2), with the exception of Ni. Nickel concentrations were greater in *Reticulitermes* frass than *Zootermopsis*, *Hylotrupes*, ptinid, and *Heterobostrychus* frass (Fig. 2). Zinc concentrations in *Reticulitermes* frass were higher than pinewood and *Zootermopsis*, *Incisitermes*, ptinid, and *Lyctus* frass (Fig. 2). Ptinid frass provided higher B concentrations than wood and all other frass (Fig. 2). Manganese concentrations were greater in *Reticulitermes*, *Zootermopsis*, and ptinid frass than wood, and was higher in *Reticulitermes* frass than all other frass (Fig. 2). *Reticulitermes* and *Zootermopsis* frass contained greater Mo concentrations than wood, with higher concentrations in *Reticulitermes* than *Incisitermes*, ptinid, *Heterobostrychus*, and *Lyctus* frass (Fig. 2). Both Cu and Fe concentrations were greater in *Reticulitermes* and *Incisitermes* frass than wood (Fig. 2). *Reticulitermes* frass provided higher Cu concentrations than four of six frass types (*Zootermopsis*, *Incisitermes*, *Hylotrupes*, ptinid) and higher Fe concentrations than all frass types, except *Zootermopsis* (Fig. 2). *Incisitermes* frass was the only pine-feeder frass that provided a lower Fe concentration than wood (Fig. 2).

Al, Co, Na, and Si are considered beneficial plant elements, a term that loosely describes elements that can promote plant growth within the context of specific taxa and environmental conditions (Pilon-Smits et al. 2009). All frass-to-pinewood and all frass-to-frass comparisons of Co concentrations were not significantly different (Fig. 3). *Reticulitermes* and *Zootermopsis* frass provided greater Al and Si concentrations than pinewood (Fig. 3). *Reticulitermes* frass contained higher Al than *Incisitermes*, ptinid, *Heterobostrychus*, and *Lyctus* frass; and higher Si than all frass types, except *Zootermopsis* (Fig. 3). Ptinid frass contained greater Na than wood and all frass types, except *Hylotrupes* (Fig. 3). The remaining elements have no known general biological function (Ba, Cr, Pb and Sr) or are considered toxic (Cd, Pb) (White and Brown 2010) (Fig. 4). Chromium was the only element that was significantly lower in all frass types than wood and was present in *Reticulitermes* frass at higher concentrations than *Zootermopsis*, *Hylotrupes*, ptinid, and *Heterobostrychus* frass (Fig. 4). Ba and Pb levels were higher in both *Reticulitermes* and *Zootermopsis* frass than wood (Fig. 4). *Reticulitermes* frass provided greater Ba concentrations than all other frass and greater Pb concentrations than *Incisitermes*, *Heterobostrychus*, and *Lyctus* frass (Fig. 4). Strontium concentrations were greater in all termite frass types than wood and higher in *Reticulitermes* frass than all other frass types, except *Lyctus* (Fig. 4). *Reticulitermes* frass provided greater Cd concentrations than wood and all other frass types, except *Lyctus* (Fig. 4). Median element concentrations that were below or just above the detection limit (<2 mg/kg) for all frass types and wood include Cd, Co, and Ni (Figs. 2–4). All 23 elements we report account for ~54.5% of the wood dry weight, with the less common elements (Al, B, Ba, Cr, Cu, Na, P, Pb, Sr, and Zn) adding ~0.022%.

Percent approximate digestibility can be interpreted as the percentage of median elemental concentrations (excluding C and N) in pinewood egested with the insect frass (Table 1). For example, the negative PAD value for Fe for *Reticulitermes* (−205) indicates that Fe is approximately 205% more concentrated in *Reticulitermes* frass than pinewood. In contrast, the positive PAD value for Fe in *Incisitermes* (81) indicates that Fe is approximately 81% less concentrated in *Incisitermes* frass than pinewood. There were 10 elements that provided positive PAD values in at least one frass type. Two elements, Cr in all frass (PAD range from 67 to 89) and Fe in *Incisitermes* frass (PAD of 81), were statistically lower than wood (Table 1, Figs. 2 and 4). All elements except Co, Cr, and Ni provided at least one negative PAD value that corresponded to a significant pairwise comparison. *Reticulitermes* frass provided PAD values < −200 in 16 of 20 elements. The frass type with the next highest number of PAD values < −200 was *Zootermopsis* with 6; followed by Ptinidae with 4, *Hylotrupes* with 3, and *Incisitermes* with none.

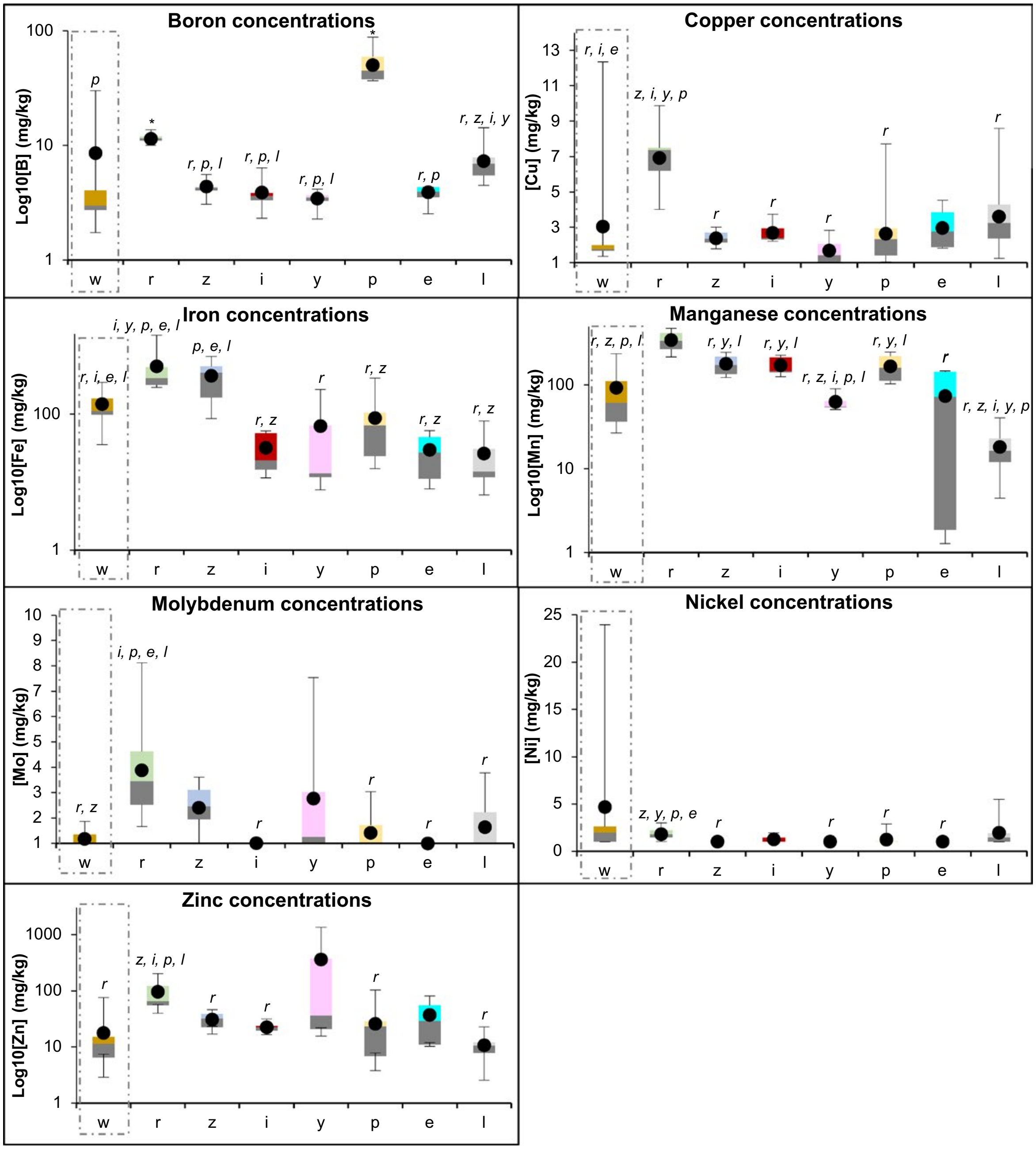

Fig. 2. Box and whisker charts showing concentrations of essential plant microelements. See Fig. 1 for symbols.

Discussion

Cellulose and lignin are the main components of wood, representing 58% to 85% of dry wood weight (Pettersen 1984). It is therefore unremarkable that carbon was the most abundant element in our frass and wood samples (Fig. 1), with %C ranges similar to that reported by Lamlom and Savidge (2003). The ability to digest cellulose has been documented in ptinids, cerambycids, lyctids, and termites, with termites exhibiting higher PAD values (Martin 1983,

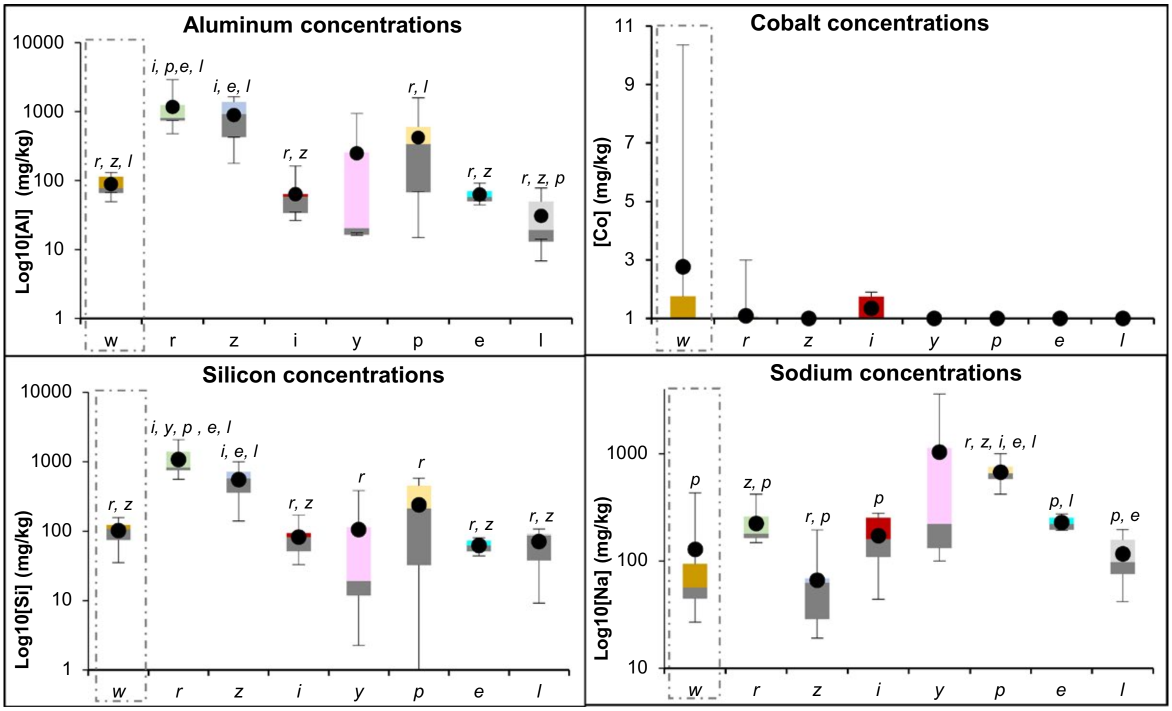

Fig. 3. Box and whisker charts showing concentrations of beneficial plant microelements. See Fig. 1 for symbols.

Kartika and Yoshimura 2013). Katsumata et al. (2007) observed that *Cryptotermes brevis* (West Indian Drywood termite) frass contained over twice the percentage of lignin (~70%) found in undigested wood (~30%), and therefore, inferred that lignin was not efficiently digested. This inefficient digestion is perhaps why our *Incisitermes* samples were the only frass type that provided higher %C than pinewood, whereas the other frass types had %C values similar to the reference pinewood (Fig. 1). Overall, the majority of PAD values in this study were negative, and therefore, not comparable to previously reported positive PAD values (Martin 1983, Slansky 1985, Grace and Yamamoto 2009). Our data are the first to examine PAD in respect of the utilization efficiencies of discrete elements. Our negative PAD values suggest that the majority of the elements in wood were somewhat "indigestible" or ingested in excess of dietary needs (Table 1). The positive PAD values we recorded for Cr in all frass types suggests that Cr may be a dietary requirement in wood-feeding insect physiology but is in short supply in comparison to elements with negative PAD values. (Table 1). The role of Cr as an essential dietary element for mammalian glucose tolerance is a current topic of debate (Anderson 1997, Vincent 2010, Bona et al. 2011). It is possible that Cr is an essential dietary element for saproxylic insects, perhaps functioning as a cofactor in the regulation of glucose or other simple sugars produced by the breakdown of cellulose. This speculated functional role of Cr is a potential research direction for insect physiology.

Concentrations of the macroelements Ca, K, Mg, N, and P were greater in *Reticulitermes* frass than three or more of the other frass types (Fig. 1). *Zootermopsis* and *Incisitermes* frass contained lower K concentrations (~5 and 11 times, respectively) than reported in the hindgut fluid of these insects (Vu et al. 2004, Fig. 1), indicating utilization of K. Contrastingly, median Mg and Ca were at least four times greater in *Zootermopsis* and *Incisitermes* frass than the hindgut concentrations reported by Vu et al. (2004), suggesting these elements were ingested in excess of dietary needs. Nitrogen is often a limiting resource for plants, and our median N concentrations in pinewood

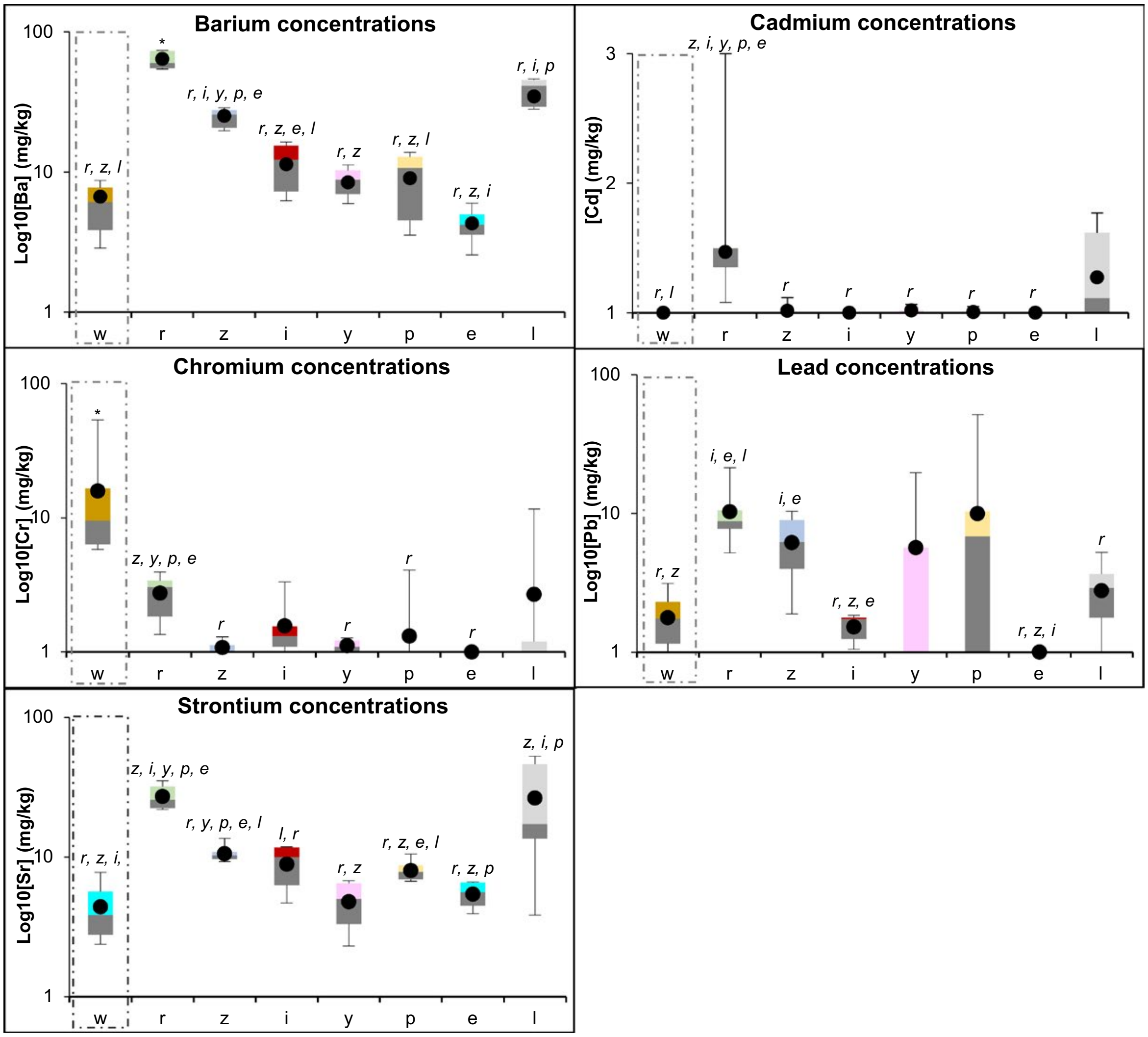

Fig. 4. Box and whisker charts showing concentrations of elements with no known physiological function (or are considered toxic). See Fig. 1 for symbols.

and *Reticulitermes* frass (Fig. 1) were similar to previously reported values (Mattson 1980, Potrikus and Breznak 1980). Nevertheless, all our frass types provided approximately twice the %N recovered from pinewood (Fig. 1), likely because of the nitrogen-fixing capabilities of termites and wood boring beetles (Suárez and Thorne 2000, Bignell et al. 2011, Ayayee et al. 2014). Similarly, Ca, K, Mg, and P, were all egested at statistically higher concentrations than wood by two or more of our pine-feeding taxa (Fig. 1), indicating the potential additive effects of saproxylic insect activity in the release of these elements from wood (Table 1). However, no further conclusions can be drawn concerning saproxylic insect frass and C, N, or P cycles without knowledge on their chemical partitioning (e.g., organic or inorganic) and the physiological processes that lead to egestion.

Micronutrients analyzed in this study included B, Cu, Fe, Mn, Mo, Ni, and Zn and all were present in pinewood at median concentrations <100 ppm, with the exception of Fe (Fig. 2). Aside from Cr, Ni was the only element that provided positive PAD values across all frass types, indicating utilization, rather than egestion, of the available stores in pinewood (Table 1). Ptinid frass provided significantly higher concentrations of B than wood and all frass types, except *Hylotrupes*

(Fig. 2), and thus may be a source of B released from CWD. Esenin and Ma (2000) concluded that cerambycid frass from their "less polluted site" contained similar Zn concentrations as wood. Cerambycid (*Hylotrupes*) frass and wood Zn concentrations were not significantly different in this study; however, our *Hylotrupes* frass Zn concentrations ranged from 15.7 to 1350 mg/kg (Fig. 2). Therefore, their role in Zn recycling remains unclear and requires further study. Cu, Fe, Mn, and Mo concentrations were higher in multiple pine-feeder frass types than wood, and *Reticulitermes* frass provided higher concentrations of all these elements than wood (Fig. 2).

The elements not yet discussed are characterized as beneficial for plants (Al, Co, Si, and Na; Fig. 3), have no known general biological function (Ba, Cr, and Sr; Fig. 4), or are considered toxic (Cd, and Pb; Fig. 4) (Fraústo daSilva and Williams 2001, Álvarez et al. 2005, Pilon-Smits et al. 2009, White and Brown 2010). Median Co concentrations were below the ICP-OES limit for all frass types and wood (Fig. 3). Sodium is an essential element, and the sodium ecosystem respiration (SER) hypothesis postulates that it limits termite activity and abundance in highly weathered inland tropical soils (Cromack et al. 1977, Kaspari et al. 2014). Only Ptinid frass provided significantly higher concentrations of Na than wood (Fig. 3). Nevertheless, median Na concentrations in all pine-feeder frass types were twice the median concentration in pinewood, except *Zootermopsis* frass. Further studies should investigate the SER hypothesis in the context of temperate forests. *Reticulitermes* and *Zootermopsis* frass provided higher Si concentrations than wood (Fig. 3) but the potential input from saproxylic insect frass may be insignificant compared to abiotic processes (Schlesinger 1997). Aluminum is classified as a beneficial plant element but can be toxic at concentrations >1350 ppm (Álvarez et al. 2005, Pilon-Smits et al. 2009). However, all our median Al values were below 1000 ppm and, therefore, potentially beneficial for certain plants (Fig. 3). Cd and Pb were noticeably higher in *Reticulitermes* frass than wood (Fig. 4), and the mechanism used by these insects to excrete heavy metals would be an interesting system for future research.

These saproxylic insect frass data also can be examined from two perspectives: (1) social vs. solitary lifestyle; and (2) association of the food resource with soil. Termites are known to share, and therefore recycle, nutrients between colony members through trophallaxis (Suárez and Thorne 2000, Bignell et al. 2011). Due to serial passage of a wood-meal, it could be expected that social insect frass would provide higher concentrations of elements than frass from solitary saproxylic insects. We examined this hypothesis using the ratio of statistically significant, negative PAD values to the total number of PAD values in each category. The PAD ratio for eusocial insects was 48% (29/60), whereas the PAD ratio for solitary insects was ~17.5% (7/40) (Table 1). Interestingly, the *Incisitermes* data were more similar to the solitary beetles than their termite kin (Table 1).

Reticulitermes was the only saproxylic taxa examined that is closely associated with the soil habitat (Jones et al. 1994, Lavelle et al. 2006, Jouquet et al. 2011), and they provided higher concentrations of 10 essential elements (N, P, Ca, K, Mg, Cu, Fe, Mn, Mo, and Zn) than pinewood (Figs. 1 and 2). *Zootermopsis* also feeds on wood in contact with the soil and provided the second highest number (6) of frass-concentrated elements (Figs. 1 and 2). These two frass types provided a PAD ratio of 60% (24/40), whereas the snag-dependent, pine-feeders (*Incisitermes*, *Hylotrupes*, and Ptinid) provided a PAD ratio of 20% (12/60; Table 1). This PAD-related association with the soil is confounded by the eusocial lifestyle, with a greater potential for food sharing and endosymbiont-host "digestion" in termites than solitary beetles. The physiological processes involved in saproxylic insect frass production are an area of forest nutrient cycling research that needs further elucidation.

Subterranean termite frass is a viscous liquid deposited on the wood food resource and gallery systems utilized by these insects (Nutting et al. 1987), *Zootermopsis* frass is a moist, barrel-shaped dropping deposited within the galleries constructed in their food source (B.T. Forschler, *personal observations*). The snag-dependent beetles—Ptinids, *Lyctus*, *Heterobostruchus*, and *Hylotrupes*—pack their galleries with powdery frass, whereas *Incisitermes* has a heavier, well-formed fecal pellet that is often ejected from infested wood (Creffield 1991). The frass in these galleries has a higher surface area to volume ratio than surrounding wood and should be more easily colonized by microbial agents of wood decay. K, Mg, Ca, and Fe, all of which are nutrients required by wood decay fungi (Ginterová and Janotková 1975), were present in higher concentrations in at least one frass type compared to wood (Figs. 1 and 2). Therefore, species that deposit nutrient rich frass within their galleries are likely facilitators of degradation by opportunistic microbial and/or fungal groups, whereas species that actively mix frass with soil are more likely to have a direct role in soil nutrient cycles. Filipiak and Weiner (2014) stated that fungi enrich wood with nutritional elements, making it a more suitable food resource for wood-feeding beetles (Buprestidae and Cerambycidae). Whether these insects facilitate fungal growth or vice versa still remains unclear.

Wood degradation, from a broader biological perspective, is an additive process that involves the efforts of bacteria, mold, stain, decay fungi, and various arthropods (Ibach 2013). Our results support that the guild of wood-feeding insects have a cumulative role in the release of microelements from CWD and conclude that saproxylic insects are ecosystem engineers that change both the physical and chemical properties of CWD, making nutritive elements available to other components of the forest ecosystem (Jones et al. 1994). While it is often assumed that subterranean termites are important for nutrient cycles, there is scant empirical evidence on how they affect soil properties in temperate systems (Neupane et al. 2015). Despite their cryptic lifestyle, the ecosystem services provided by saproxylic insects should not be overlooked but included in future nutrient cycling studies.

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