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1 **Mercury species in the nests and bodies of soil-feeding termites, *Silvestritermes* spp.**
2 **(Termitidae, Syntermitinae), in French Guiana**

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20

21 **Abstract**

22 Mercury pollution is currently a major public health concern, given the adverse effects of mercury on wildlife and humans. Soil plays an essential role in
23 speciation of mercury and its global cycling, while being a habitat for a wide range of terrestrial fauna. Soil fauna, primarily soil-feeding taxa that are in intimate
24 contact with soil pollutants are key contributors in the cycling of soil mercury and might provide relevant indications about soil pollution. We studied the
25 enrichment of various mercury species in the nests and bodies of soil-feeding termites *Silvestritermes* spp. in French Guiana. Soil-feeding termites are the only
26 social insects using soil as both shelter and food and are major decomposers of organic matter in neotropical forests. Nests of *S. minutus* were depleted in total
27 and mobile mercury compared to nearby soil. In contrast, they were enriched 17 times in methylmercury. The highest concentrations of methylmercury were
28 found in body of both studied termite species, with mean bioconcentration factors of 58 for *S. minutus* and 179 for *S. holmgreni* relative to the soil. The
29 assessment of the body distribution of methylmercury in *S. minutus* showed concentrations of 221 ng.g⁻¹ for the guts and even higher for the gut-free carcasses
30 (683 ng.g⁻¹), suggesting that methylmercury is not confined to the gut where it was likely produced, but rather stored in various tissues. This enrichment in the
31 most toxic form of Hg in termites may be of concern on termite predators and the higher levels in the food chain that may be endangered through prey-to-predator
32 transfers and bioaccumulation. Soil-feeding termites appear to be promising candidates as bio-indicators of mercury pollution [in soils of neotropical rainforest](#)
33 [ecosystems](#).

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36 Keywords: soil-feeding termites; Syntermitinae; soil mercury; mercury methylation; bio-concentration

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39 Nests of the soil-feeding termites *Silvestritermes* spp. and the tissues of the inhabiting worker caste are hotspots of methylmercury, but are depleted in total and
40 mobile mercury.

41

42 **Introduction**

43 Mercury (Hg) is a pervasive neurotoxic heavy metal that has been introduced in diverse ecosystems through various mechanisms including natural erosion of the
44 parent bedrock and anthropogenic activities (Science for Environment Policy, 2017). Living organisms are generally more tolerant of inorganic forms of Hg than
45 its organic forms. Methylmercury (MeHg), a major form of organic mercury [in food chain \(Benford et al. 2018; Rice et al. 2014\)](#), is membrane-permeable, thiol
46 reactive [\(Clarkson & Magos 2006\)](#) and with a long biological half-life [\(Jo et al. 2015\)](#). It is therefore especially toxic and tends to accumulate in animal tissues,
47 especially in longer-lived organisms and in predatory species (Scheuhammer et al. 2007). One of the main sources of MeHg in biota is the biotransformation of
48 inorganic Hg to MeHg by microbial methylators carrying the *hgcAB* gene cluster that is required for Hg methylation in anoxic conditions (Parks et al. 2013;
49 Poulain and Barkay 2013; Gilmour et al. 2013). Aquatic microorganisms have been widely recognized as Hg biotransformers and, consequently, Hg methylation
50 has usually been associated with aquatic food webs, sometimes extending to terrestrial invertebrates feeding on aquatic organisms (Cristol et al. 2008; Becker et
51 al. 2018; Bartrons et al. 2015; Saxton et al. 2013). There is now, however, a paradigm shift with increasing evidence of Hg biotransformation by various
52 terrestrial invertebrates, amongst [which earthworms \(Zhang et al. 2009; Rieder et al. 2011; Rodríguez Álvarez et al. 2014; Álvarez et al. 2018; Rieder et al. 2011;](#)
53 [Rodríguez Álvarez et al. 2014; Kaschak et al. 2014\)](#) and isopods [\(Jereb et al. 2003\)](#). [This extends the process beyond aquatic and wetland ecosystems \(reviewed](#)
54 [by Mahbub et al. 2017\)](#).

55 [Nevertheless, the potential for Hg methylation significant differ between invertebrates in general, and between arthropod taxa in particular as shown by](#) Podar and
56 colleagues (Podar et al. 2015) [who found that only 4 metagenomes carried the hgcAB gene cluster out 27 metagenomes for arthropod microbiomes. Moreover,](#)
57 [most studies demonstrating methylation capability in terrestrial invertebrates have used diets spiked with Hg.](#) There are few studies addressing the effect of
58 mercury contamination on terrestrial biota in natural conditions and even fewer for soil-inhabiting social insects which have high population densities in restricted
59 habitats. In the invasive red fire ant (*Solenopsis invicta*), high levels of total mercury typical of aquatic insects have been reported, with even higher levels in
60 reproductive males (Helms & Tweedy 2017). They only studied total Hg, but authors suggested that these high levels were due to predation on aquatic fauna with
61 high levels of MeHg, rather than to environmental exposure to inorganic mercury. [Besides ants, the production of MeHg by the](#) wood-feeding Australian termite
62 [Mastotermes darwiniensis has been reported from a feeding experiment with an artificial diet spiked with Hg](#) and cultures of the gut-derived sulfate-reducing
63 bacteria isolates showed Hg methylation activity (Limper et al. 2008). We are not aware of any study on the effect of soil Hg on soil-living social insects that are
64 primary consumers that are not only directly exposed to soil Hg, but also interact with Hg through the consumption and biotransformation of Hg-containing
65 substrates.

66 Termites are [fully social insects living in highly populated colonies. Besides the brood \(eggs and larvae\), termite colonies include various castes that are devoted](#)
67 [to different tasks such as reproductives \(kings, queen, nymphs\), and sterile castes \(workers and soldiers\) \(Eggleton, 2011\). The foraging activities are](#)
68 [accomplished only by the worker caste. The majority of termites are wood-feeders ie](#) feed on woody materials at various levels of humification (Donovan et al.
69 2001; Eggleton and Tayasu, 2001). [However, about one-third of known termite species are soil-feeders, feeding on mineral soil with little recognizable plant](#)
70 [material \(Donovan et al. 2001; Inward et al. 2007\). Nests of these soil-feeding termites are built by workers using firm amalgams of soil from the 10 top cm and](#)
71 [feces. The enrichment in organic matter and in small-size particles such as clay and limon relative to the soil from the nearby environment is one of the salient](#)

72 [features of the nest materials of soil-feeding termites. These are](#) particularly abundant and diverse in tropical rain forests, appearing as one of the main
73 contributors in soil processing and humification. Through their soil consumption and burrowing activities, they have a major effect on soil physical, chemical and
74 biological properties, which, in turn, affects the cycling and speciation of elements, including trace metals, in the soil (Brauman 2000; Donovan et al. 2001;
75 Sarcinelli et al. 2009, 2013; de Lima et al. 2018). Their effect on soil properties could be compared to that of earthworms whose effects on heavy metals in soil
76 have been more widely studied. However, soil-feeding termites are ecologically distinct from earthworms in various ways: unlike earthworms, they are social and
77 thus live in densely populated colonies, they have longer-lasting biostructures than worm casts and burrows and they have a more complex gut structure hosting a
78 more complex microbiota.

79 [Soil-feeding termites are the dominant feeding group of termites in various Amazonian rainforest ecosystems. In various oxisols of these ecosystems](#) the soil
80 mercury concentration is between 0.25 and 0.5 $\mu\text{g}\cdot\text{g}^{-1}$ (Lechler et al. 2000; Richard et al. 2000), well above the normal levels in soil (0.05 to 0.08 $\mu\text{g}\cdot\text{g}^{-1}$) reported
81 by the World Bank (1999). They also exceed the limits relative to soil organic matter (SOM) (0.5 $\mu\text{g}\cdot\text{g}^{-1}$ SOM) proposed by de Vries and colleagues (de Vries et al.
82 2007) and relative to the soil (0.13 $\mu\text{g}\cdot\text{g}^{-1}$ of dry weight soil) proposed by Tipping and colleagues (Tipping et al. 2010). The topsoil of these ecosystems is
83 therefore subject to a generalized, chronic excess of Hg, although the background mercury level (in the bedrock) is significantly lower (0.020 - 0.1 $\mu\text{g}\cdot\text{g}^{-1}$) (Aula
84 et al. 1993; Pfeiffer et al. 1993). This Hg overload is believed to arise mainly from atmospheric Hg deposition over several millions of years, which has been
85 particularly high from the end of the 15th century (Roulet and Grimaldi, 2001; Obrist et al. 2018). In forest soils on the French Guiana plateau, there is a high
86 abundance and diversity of soil-feeding termites, including many endemic species, which may indicate that this chronic exposition to these high levels of mercury
87 may not be directly harmful to these termites. Some animals can thrive at contamination levels generally considered to be lethal by sequestering and
88 accumulating the toxic agent in non-essential tissues (Lanno et al. 2004; Mahbub et al. 2017). Nevertheless, they may still pose a risk for the biota by exposing
89 their predators and the upper levels of the food chain to high levels of mercury in their food. For assessing this risk, Hg storage in animal tissues is more relevant
90 than the sensitivity to mercury exposure.

91 Our study addresses the processing of soil mercury by soil-feeding termites from the genus *Silvestritermes*. We compared the concentrations of various mercury
92 species in the soil around the nest harvested by the termites and the concentrations in their nests. *Silvestritermes* is an abundant genus of soil-feeding termites in
93 the neotropical forests, belonging to the subfamily Syntermitinae (Termitidae family) that is endemic to this region. [As for organic mercury, we compared](#)
94 [concentrations of methylmercury](#) in the nest, termite bodies and [termite](#) body parts [to](#) those of [the nearby soil](#). We discuss the concerns raised by Hg
95 biotransformation by termites considering the role of termitophagy in the food webs.

96 **Material and Methods**

97 **Sampling and sample processing**

98 The main sampling site was at Crique Combat, which is an area of 1 km² covered by a primary rainforest, near the village of Cacao (Figure S1, Supplementary
99 information). The soil in this area is well-characterized since this site has hosted several research programs (such as “Mercury in Guyana” (2002-2007) run by
100 CNRS (French National Research Organization)) and several thesis projects (Guedron et al. 2009; Harris-Hellal et al. 2011). Six colonies of the soil-feeding
101 termite *Silvestritermes minutus* and four colonies of *S. holmgreni* were randomly sampled from the oxisol (USDA Soil Taxonomy) at the top of the slope, with a

102 minimum distance of 20 m between them. [Both species build well-defined nests that are spherical to ellipsoid structures emerging a few centimeters above the](#)
103 [ground level. For *S. minutus*, nests were generally built on plants sprouts, thus penetrated and consolidated by a network of plants roots for mature colonies \(see](#)
104 [Fig. S2\). As for *S. holmgreni*, their nests are generally built laterally against the basis of tree trunks.](#)

105 Nests were partially broken to collect a representative selection of the nest material, with termite individuals inside. A composite soil sample was collected from
106 nearby each nest. This was a mix of four cores from the 0-10 cm layer where soil-feeding termites are mainly found (Brauman 2000), at a radius of 2 m from the
107 nest. Then, the termites were separated from the nest material using plastic tweezers. Only workers were collected for mercury analyses: these are the only caste
108 feeding directly on the soil, unlike the other castes which are fed by workers. The nest material and the soil were hand sorted to remove roots and pebbles and
109 then crushed. All samples (workers, nest material and soil) were stored in sterile, trace metal-free Falcon tubes and frozen at -20°C. Then, they were transported
110 in dry ice to mainland France, where they were frozen at -80°C until analysis.

111 Termites from the second sampling site near Petit Saut ([S. minutus only](#)) were used to analyze the MeHg distribution in their bodies. Whole colonies were
112 collected [by disrupting at the base of nest the roots that maintain them tied to the ground \(see fig. S2B\). They were](#) shipped to mainland France. Nearby soil
113 samples were collected and transported as described above. Samples were processed within two days of arrival at the laboratory. The guts were removed from the
114 workers using fine sterile tweezers and the gut-free carcasses, with the head and the legs, were kept as separate samples. The guts and gut-free carcasses for each
115 colony were pooled in separate sterile microtubes. All the samples (soil, guts and gut-free carcasses) were stored at -80°C until analysis.

116 [Five to 10](#) soldiers were collected [from](#) each colony for the identification of the termite species. The species identity was checked from morphological criterion
117 and by DNA barcoding, based on the cytochrome oxidase I gene. For DNA extraction from the head of soldiers, PCR amplification and sequencing, the method
118 used by Fougeyrollas and colleagues was applied (Fougeyrollas [et al.](#) 2018).

119 **Total mercury and mobile mercury analyses of the nest material and the nearby soil**

120 Total mercury (THg) was determined using an atomic absorption spectrometer AMA-254 (Advanced Mercury Analyser, Altec, Ltd., Czech Republic) following
121 the protocol described by Sysalová [et al.](#) 2013. This measures trace levels mercury in samples directly without pre-treatment by mercury extraction. One hundred
122 milligrams of soil or nest material were placed in a nickel boat and introduced into the spectrometer. After drying at 120°C, the samples were combusted at 550°C
123 for 150 sec in an oxygen-saturated atmosphere transforming the total mercury into elementary mercury (Hg⁰) which is readily adsorbed onto a gold-trap. The
124 mercury was then released by heating the gold-trap to 450°C and quantified by atomic adsorption spectrometry at a wavelength of 253.7 nm.

125 The mobile, toxic mercury (MHg) fraction, comprising soluble inorganic Hg and alkyl-Hg forms (methyl and ethyl Hg), was extracted using acidic ethanol with
126 1:1 (v/v) 2% hydrochloric acid and 10% ethanol (Han [et al.](#) 2003; Reis [et al.](#) 2010; Frentiu [et al.](#) 2013). Except for the initial sample weight (500 mg in this
127 study), the mercury was extracted using the protocol described by Frentiu [et al.](#) 2013. All samples were then filtered through PTFE filters (0.45 µm) and acidified
128 to a pH of 1. This mercury fraction was measured using the AMA-254 as described above for the solid samples, manually injecting 200 µl of the filtrate.

129 The detection limit ranged from 0.01 to 500 ng of Hg in a sample. Dilutions from a 1000 mg.l⁻¹ mercury standard solution (Hg PlasmaCAL, SCP Science) were
130 used for calibration. [The data acquisition was driven by AMA 254 V5.0.2.4 software . The routine quality assurance metrics included in the standardized](#)
131 [analytical method were fulfilled by all samples. The mercury content of the standard reference materials namely LGC6139 \(River clay sediment-Metals\) and](#)

132 [BCR 277R \(Estuarine sediment\) was recovered within certified limits: 1.2 \(\$\pm 0.05\$ \) \$\mu\text{g}\cdot\text{g}^{-1}\$ and 0.128 \(\$\pm 0.017\$ \) \$\mu\text{g}\cdot\text{g}^{-1}\$ respectively. Likewise, triplicated samples](#)
133 [passed the method criteria of <20% RSD \(Relative Standard Deviation\) for liquids samples and <5 % RSD for solids samples.](#)

134 **Analysis of methylmercury in termites, nests and soil samples**

135 MeHg concentrations were measured at the Wisconsin State Laboratory of Hygiene (WSLH), by the Inorganic Chemistry Unit of the Environmental Health
136 Division. After extraction by grinding freeze-dried samples with an agate mortar and pestle and digesting known amounts of matter with KOH-methanol, MeHg
137 was measured by aqueous ethylation, purge and trap, desorption and Cold Vapor Atomic Fluorescence Spectrophotometer (CVAFS, Brooks Rand model III)
138 following a standard procedure (ESS INO METHOD 545.1 rev. 4). [All samples passed the routine assurance quality metrics included in this standardized](#)
139 [analytical method. For example, both NRCC standard materials \(National Research Council Canada\), TORT-3 and DORM-3 were recovered within the certified](#)
140 [limits 105% and 119% respectively. Also, matrix spikes on samples were recovered at 108% and 95.4%. Finally, duplicated samples passed the acceptance](#)
141 [criteria of <35% RSD. On a dry weight basis, duplicate 1 was 481 and 383 \$\text{ng}\cdot\text{g}^{-1}\$ and duplicated 2 was 2.08 and 2.83 \$\text{ng}\cdot\text{g}^{-1}\$ dry weight.](#)

142 **Statistical analyses**

143 The statistical analyses were performed using the Statgraphics software (Centurion XVI, Sigma Plus, France). For the concentrations of total (THg), mobile Hg
144 (MHg) and the ratio of MHg to THg, the data was not normally distributed (Shapiro-Wilk test at 95% confidence). Therefore, the non-parametric Kolmogorov-
145 Smirnov test was used to assess the significance of the differences in concentration, with $p < 0.05$. MeHg concentrations were, however, normally distributed.
146 Differences between samples of each termite species were analyzed using one-way ANOVA. Correlations between the various mercury fractions were tested
147 using Spearman's rank correlation ($p < 0.05$) and the correlation curves were constructed using *ggplot2* and *ggpubr* packages in R version 3.4.4.

148 **Results**

149 **Concentration of total (THg) and mobile Hg (MHg) in *S. minutus* nests and the nearby soil**

150 THg concentration was not statistically different between nests and the nearby soil ($p=0.149$) (fig. 1A). The THg concentration in the nests appear to be related to
151 the concentration in the nearby soil as shown by the high positive correlation between THg concentrations in the two compartments ($R=0.93$; $p=0.007$) (fig. 2A).
152 The MHg concentration in the nest material was significantly lower than in the soil (fig. 1B). The MHg concentration was less variable between the nest materials
153 than between the soils. Unlike THg, the MHg concentrations in the nest materials were not correlated with that in the nearby soil. However, the MHg
154 concentration in both the nest and the soil seems to be related to THg concentration ($R=0.76$; $p=0.0041$) (fig. 2B). The mobile mercury percentage (relative to
155 THg) was significantly lower in the nest than in the soil ($p=0.037$) (fig. 1C) and was correlated with the MHg concentration ($R=0.85$; $p=0.0005$) rather than with
156 the THg concentration ($R=0.38$; $p=0.22$) (fig. 2 C & D).

157 **MeHg concentration in soil, nests and the termite whole bodies of *S. minutus* and *S. holmgreni***

158 At the Crique Combat site, [the concentration of MeHg was lower in the nearby soil than in all other samples of *S. minutus*, covering](#) only a small fraction of the
159 THg ($0.18 \pm 0.10\%$) (Table 1). [For this termite species, unlike THg and MHg, the MeHg concentrations in the nest material were far higher than in the nearby](#)
160 [soil and covered a significantly higher fraction of THg \(\$4.73 \pm 4.42\%\$ \).](#) MeHg concentration was independent of THg or MHg concentrations according to the

161 correlation tests. The bodies of *S. minutus* had higher concentrations of MeHg than the nearby soil and nests. [Since termite workers reside either in the soil \(for](#)
162 [the foraging tasks\) or in the nest \(for the social and building tasks\), the bio-concentration factor \(BCF\) of MeHg in the termite body was calculated relative to](#)
163 [both habitats. The resulting BFC was much higher relative to the nearby soil \(\$57.84 \pm 25.85\$ \), but remained substantial high](#) relative to the nest (3.76 ± 1.61).
164 [While the body concentration of MeHg was uncorrelated with the concentration in the soil, it was significantly correlated positively with](#) the concentration in the
165 nest ($R=0.89$).

166 For the congeneric species, *S. holmgreni*, living in sympatry with *S. minutus*, [except the lack of statistical difference between MeHg in the soil and in the nest, the](#)
167 results were similar, with even a higher BCF [in the bodies](#) relative to the nest and to the soil.

168 The second set of samples, from Petit Saut, was analyzed to determine whether the high amounts of MeHg in the body of termites were restricted to the gut or
169 widespread through the tissues. The MeHg concentration was measured for the whole gut (accounting for over two-thirds of the termite body weight) and for the
170 gut-free carcasses separately, as well as for the nest and the nearby soil (Table 2). The MeHg concentration of the nearby soil was below the detection limit of the
171 analysis method while in the nests, it was in the same order of magnitude as [in nests from Cacao](#). The MeHg concentration in the termite body parts was much
172 higher than in the soil and nests. The highest concentrations of MeHg were found in the termite carcasses. The BCF in the body parts could not be calculated
173 relative to the soil, but was very high relative to the nest material both for the termite guts and for the gut-free carcasses.

174 Discussion

175 In this study, we assess various mercury forms in nests and the bodies of soil-feeding termites. Several studies have pointed out the effects of termite activities on
176 soil physical and chemical properties and, thereby, on soil heterogeneity at the landscape level (Jouquet et al. 2011). These effects on soil properties are
177 characteristic of all termite feeding groups, even including the majority of termites feeding on above ground litter and wood material. However, soil-feeding
178 termites consume, partially or exclusively, soil (Inward, Vogler and Eggleton 2007; Eggleton and Tayasu 2001) in a similar way to earthworms, and have a more
179 significant contribution (Brauman 2000; Donovan et al. 2001; Sarcinelli et al. 2009, 2013; de Lima et al. 2018). This is particularly true in neotropical rainforests
180 where they are among the most [representative feeding-groups of termites](#) in various forest habitats (Ackerman et al. 2009; de Sales Dambros et al. 2013; de Souza
181 and Brown 1994; Davies et al. 2003). [This is the case in](#) French Guiana where up to 73 species [of termites](#) are soil-feeding out of 100 species locally recorded
182 (Davies et al. 2003).

183 The THg concentration in the soil at Crique Combat averaged $0.383 \mu\text{g.g}^{-1}$ of dry soil, which is within the range of concentrations recorded in various Amazonian
184 oxisols ($0.250\text{-}0.500 \mu\text{g.g}^{-1}$) (Lechler et al. 2000; Richardet et al. 2000) and, in particular, previously recorded for oxisols from the same study site (Guedron et al.
185 2009; Da Silva et al. 2016). This THg concentration is well above normal levels in soil (0.05 to $0.08 \mu\text{g.g}^{-1}$) reported by the World Bank (1999). It also exceeds
186 proposed limits relative to SOM ($0.5 \mu\text{g.g}^{-1}$ SOM, de Vries et al. 2007), as the SOM in this horizon is around $5.6 \pm 0.6\%$ dry soil (Da Silva et al. 2013), and
187 relative to the soil ($0.13 \mu\text{g.g}^{-1}$ dry soil, Tipping et al. 2010). [Soil-feeding behavior in this case may result in a handling of huge amounts of mercury by termites.](#)
188 [Indeed, a humid savannah ecosystem, soil-feeding termites from the genus *Cubitermes* were reported to ingest annually up to \$4.5 \text{ kg m}^{-2}\$ \(Lavelle et al. 1997\).](#)
189 [Likewise, a daily consumption of soil ranging from \$0.72\$ to \$0.911 \text{ mg}\$ of soil has been reported for another species *Cubitermes* \(Okwakol, 1980\), that is almost the](#)

190 [equivalent of its own weight \(≈10 mg\). Though to our knowledge, the daily consumption of soil was not yet reported for the *Silvestritermes* species understudy,](#)
191 [the fate of the Hg that is ingested all the time with soil emerged as a crucial question.](#)

192 The THg concentration for each nest was below that of the nearby soil, although this was not statistically significant, but there was a significant positive
193 correlation between THg concentrations in the soil and in the nest material, indicating that the THg concentration in the nests of soil-feeding termites could be
194 predicted from the THg concentration in the soil.

195 The toxicity to biota of heavy metals in general, and of mercury in particular, depends on the bioavailable soluble fraction rather than on the total concentration.
196 Extracting Hg with an acid ethanol solution can be used to assess the mobile, toxic fraction comprising of soluble inorganic and alkyl Hg species (Han et al.
197 2003). We anticipated that soil processing by the termites should release adsorbed mercury, and therefore, lead to a higher fraction of mobile mercury in the nest
198 than in the soil. Unexpectedly, the MHg concentration and its proportion of THg were significantly lower in the nests than in the nearby soil. Soil processing did
199 not therefore increase the MHg concentration, unless the mobilized Hg was then converted into volatile or immobile organic forms.

200 As far as we know, no data are available on the effect of termites' nest building activities on the mercury naturally present in the soil. [As stated above, nests of](#)
201 [soil-feeding termites are a fine mixture of soil and fecal matter and, as such, are not strictly comparable with true feces of other arthropods.](#) [Various factors related](#)
202 [either to the foraging and building behavior or to mercury biotransformation after ingestion could explain this decrease in the nests, especially for the MHg](#)
203 [fraction. The first possibility is](#) that soil-feeding termites preferentially select soil fractions with less affinity for Hg during harvesting. The question of food
204 particle selection by soil-feeding termites remains unanswered (Brauman 2000; Brauman et al. 2000), but their nests are commonly enriched in organic matter
205 (OM) and in small-size particles such as clay (Brauman 2000; Brauman et al. 2000; Fall et al. 2001; Sarcinelli et al. 2009, 2013; Ngugi et al. 2011; de Lima et al.
206 2018). Strangely, both SOM and clay are considered to have high affinity for, and are often positively correlated with, soil Hg (Gabriel and Williamson 2004;
207 Róžański et al. 2016). Particle selection does not, therefore, seem to explain the low mercury concentration in the nests. The lower concentration of THg and,
208 especially, MHg in the nests could be related to the microbial transformation of soil Hg. From ingestion by the termites, up to deposition on the lining of nest, the
209 soil moves through various oxic and redox conditions (Brune et al. 1995; Brune and Friedrich 2000; Kappler and Brune 2002) compatible with the microbial
210 transformation of Hg(II), either by dissimilatory reduction into elemental Hg (Hg⁰) or by methylation into MeHg. The reduction of Hg(II) into volatile Hg⁰ is
211 performed by bacteria carrying the *mer* operon is the most common Hg dissimilatory pathway (Boyd & Barkay 2012). It operates under aerobic conditions that
212 are found in various gut sections (fore-gut, mid-gut and the terminal subsections of the hind-gut) and even at the periphery of the paunch that is believed to be
213 internally anaerobic (Brune & Friedrich 2000). Moreover, it is possible that Hg is reduced under anaerobic conditions through constitutive pathways whose
214 mechanisms are less well documented (Wiatrowski et al. 2006). Anoxic conditions are found mainly in the paunch that is the largest part of the termites' gut. To
215 the best of our knowledge, this process has not yet been explored in termites. Although the conditions in the gut are potentially favorable to Hg reduction and
216 volatilization, its occurrence should be substantiated by measuring mercury fluxes and testing for the presence of the genes or microorganisms involved.

217 Methylation of Hg to MeHg was the other possible pathway explaining the depletion of inorganic Hg in the nests since the MeHg concentration increased as MHg
218 and THg decreased, MeHg concentrations being on average 17 times higher in the nests than in the soil. This enrichment could hardly be explained by the bio-
219 concentration of the small amounts of MeHg from the ingested soil. Additionally, part of the ingested MeHg might have been assimilated or demethylated during
220 the gut transit (Nolde et al. 2005). This enrichment is probably, therefore, the result of *de novo* synthesis of MeHg through Hg-methylation by the termites'

221 microbiota during soil processing. This hypothesis is supported by the MeHg concentration in the termites which was several orders of magnitude greater than in
222 the soil or nests. The MeHg concentrations in the body were similar for *S. minutus* and the congeneric species *S. holmgreni* but the latter had even higher average
223 BCFs values relative to the soil and relative to the nests suggesting that the high MeHg enrichment in termites could be a widespread phenomenon among
224 Guyanese soil-feeding termites. The mercury methylation potential of termites was first reported for *Mastotermes darwiniensis*, a wood-feeding species endemic
225 to Northern Australia (Limper et al. 2008). Unlike soil-feeding termites with purely prokaryotic microbiotas, *M. darwiniensis* also hosts protozoa (like all lower
226 termites), and is the sole extant member of the Mastotermitidae family, the most basal in the termite phylogeny. As well as the differences between termite taxa,
227 the experimental approach was different. The Hg-methylation potential of *M. darwiniensis* was tested in a short-term experiment (14 days) based on an artificial
228 diet (sawdust) spiked with much higher Hg concentrations (25-250 $\mu\text{g}\cdot\text{g}^{-1}$ HgCl_2) than in the soil of our study. The resulting MeHg concentrations in the termites
229 were in the same range as in our study (15.9-53.8 $\text{ng}\cdot\text{g}^{-1}$ DW). Although the biological traits of the taxa may be different, the similar enrichment in MeHg in the
230 body with much lower mercury concentrations under natural conditions may suggest that the length of exposure may significantly affect termites' accumulation
231 of mercury in their bodies. [Soil-feeding termites are also known to re-ingest fresh nest material \(Brauman et al. 2000\), which could contribute to this body](#)
232 [accumulation of MeHg, given its higher concentrations in the nest relative to the soil.](#)

233 MeHg is the most problematic form of mercury [for living organisms. Due to its affinity with thiol groups \(R-SH\) and selenol groups \(R-SeH, that easily replace](#)
234 [thiol groups in amino-acids\), MeHg is readily binds to cysteine, tripeptide glutathione and cysteine moieties of proteins and enzymes \(Wang et al. 2012\) that are](#)
235 [structurally and functionally pivotal in biological systems. As a result, MeHg is](#) readily adsorbed into animal tissues and bioaccumulated throughout food chains
236 (Gaur et al. 2017). The distribution of MeHg in the termite body was assessed to establish whether MeHg was limited to the gut content, and, therefore, likely to
237 be transitory, or diffusely and durably stored within termite tissues. The MeHg concentrations in the guts and gut-free carcasses from Petit Saut were much higher
238 than those in the soil and nests, in agreement with the higher concentration in the whole workers from Crique Combat. However, both body parts contained
239 substantially higher MeHg levels than the whole workers from Crique Combat. MeHg concentrations were not directly measured in the whole body, but the
240 higher concentrations in both body parts clearly indicate that workers from Petit Saut had higher whole body concentrations. The inorganic forms of mercury
241 were not measured in Petit Saut. However, the MeHg concentrations in the nests, which, for Crique Combat, were closely correlated with the MeHg concentration
242 in the body, was of the same order of magnitude as for nests from Crique Combat and soil MeHg concentrations were even lower than in soils from Crique
243 Combat. More in-depth investigation is therefore needed to explain this difference between sites in MeHg concentrations in the termite bodies.

244 The highest average concentrations of MeHg were found in worker gut-free carcasses, rather than in the gut, but the difference was not statistically significant.
245 Moreover, the gut samples were mixtures of the food bolus and the gut-wall tissues, and as the MeHg concentration was lower in the nest which is (at least partly)
246 constructed with feces, MeHg in the gut-wall tissue might have been underestimated. The main conclusion from the high MeHg concentration in the carcasses
247 was that MeHg was not confined to the gut bolus and wall, but distributed through the termite tissues. Gut-free carcasses were also mixtures: integuments
248 (cuticle), heads and legs which are more sclerotized body parts. [In the Isopod *Porcellio scaber*, a higher proportion of MeHg \(relative to the total MeHg or THg\)](#)
249 [was recorded in the residue and attributed to the high affinity of MeHg for the sylvhydryl \(-SH\) groups of some compounds involved in cuticle sclerotization](#)
250 [\(Bittner 2006\). The high proportion of sclerotized parts \(head and legs\) in these samples might explain the association of MeHg with the carcasses in our study.](#)

251 Finer analyses, separating the gut wall from the content, and separating the various parts of the carcass, are, therefore, needed to shed light on the contribution of
252 each part.

253 There is a growing body of data for THg concentrations for various arthropods under diverse conditions of mercury pollution but there is less data about the
254 concentration of organic forms of mercury in terrestrial arthropods, and much less in social insects. The concentrations of MeHg measured here in termite tissues
255 are much higher than those of THg reported for various ants, including invasive fire ants (*Solenopsis invicta*) for which the concentrations were considered to be
256 significantly high (Helms & Tweedy 2017). The lack of information about the THg concentrations in the ants' habitat limits any comparison. MeHg
257 concentrations in the bodies of various terrestrial arthropods at the same trophic level (primary consumers) have often been lower than those reported here for
258 termites. This is the case for herbivorous insects (*Locusta migratoria manilensis*, *Acrida chinensis*) (1-12 ng.g⁻¹) (Zheng et al. 2008), for pill bugs and ground
259 beetles (Ortiz et al. 2015) in Hg-polluted environments. However, the MeHg concentrations within the range we found in termites was reported for the sap-
260 feeding cicada, *Cryptotympana atrata*, in a polluted environment, probably due to a higher degree of exposure related to the longer lifespan and to a long
261 underground larval stage (4-5 years) during which the larvae feed on the Hg-rich sap from roots (Zheng et al. 2010). MeHg concentrations similar to or higher
262 than those measured here in termites are fairly common in aquatic or wetland arthropods (Ackerman et al. 2010; Bates & Hall 2012) due to high Hg-methylation
263 in these environments, as well as in terrestrial predators such as spiders, Odonata, centipedes and scorpions (Bartrons et al. 2015; Bates & Hall, 2012; Ortiz et al.
264 2015; Tavshunsky et al. 2017; Tsui et al. 2014).

265 As well as the high MeHg concentration in the termites reported here, there is a large body of evidence supporting the hypothesis that termites can methylate the
266 Hg naturally present in ingested food. The first argument is that sulfate reducing bacteria (SRB), which are the best-known group of Hg-methylators, are
267 widespread in termite microbiotas from wood-feeding lower termites (Trinkerl et al. 1990; Kuhnigk et al. 1996; Fröhlich et al. 1999; Dröge et al. 2005; Limper et
268 al. 2008) to higher termites (protozoa-free) including soil-feeders (Brauman et al. 1990; Kuhnigk et al. 1996). SRB from the genus *Desulfovibrio*, about 26% of
269 known bacterial methylators (Gilmour et al. 2018), and those from the genus *Desulfarculus*, are highly conserved across termite microbiotas (Abdul Rahman et
270 al. 2015), including in soil-feeders (Brauman et al. 1990; Kuhnigk et al. 1996). Additionally, several SRB strains isolated from termite guts can methylate Hg(II)
271 *in vitro* (Kaschak et al. 2014; Limper et al. 2008), including a strain from a soil-feeding termite *Cubitermes* sp. (Kaschak et al. 2014). The second argument is that
272 the *hgcAB* gene cluster for Hg-methylation (Parks et al. 2013; Poulain and Barkay 2013) has been found in various higher termite metagenomes, including those
273 of soil-feeding species (Podar et al. 2015; Martín-Doimeadios et al. 2017). Methanogenic archaea, including Hg methylators (Gilmour et al. 2018), are also very
274 prevalent in termite microbiotas (reviewed by Brune, 2018) including those of soil-feeders (Ohkuma et al. 1999; Donovan et al. 2004). Since the bacterial and
275 methanogenic archaeal methylators probably share the same micro-environment, the syntrophic interactions for Hg-methylation that are probably widespread in
276 complex systems (Yu et al. 2018) may also occur in the termite gut. All this evidence that termites are potentially Hg methylators, strongly suggest that the high
277 MeHg concentrations in the termite bodies arise from the activity of Hg-methylators in the gut microbiota. Additional experiments comparing the extent and
278 diversity of the *hgcAB* gene cluster in the gut, as a predictor of Hg-methylation potential, with the MeHg concentrations in the body tissues under various levels
279 of mercury pollution are needed for a better understanding of the role of these microbial methylators.

280 **Conclusion**

281 To our knowledge, this is the first study addressing the effect of termites on the fate of soil mercury under natural conditions. Nests of the soil-feeding termites
282 that significantly contribute to the heterogeneity of soils in tropical forests are depleted in inorganic mercury while the environmental levels of mercury were
283 rather high. The turnover of their nests (erosion, colony death) reduces soil mercury concentrations at local scales. Organic mercury shows the opposite pattern
284 with the nests and their inhabiting termites being hotspots of MeHg. [These](#) high MeHg concentrations found, especially in termite tissues, can be a concern,
285 especially in heavily contaminated sites. This preoccupation is further justified by the findings that MeHg enrichment seems to be widespread within soil-feeding
286 termites that are among the most common termites in neotropical rainforest ecosystems (Ackerman et al. 2009; de Sales Dambros et al. 2013; de Souza and
287 Brown 1994; Davies et al. 2003). Moreover, termites are either opportunistic or specialized prey for various invertebrates such as many species of ants
288 (Buczowski & Bennett 2007; Eggleton 2011; Wen et al. 2017), spiders (Pekár & Toft 2015) and vertebrates like anteaters, pangolins, aardvarks, etc. (Eggleton
289 2011). With prey-to-predator transfer and bioaccumulation of MeHg, [the health of](#) termite predators, especially those feeding regularly on termites from heavily
290 polluted sites [may be placed at risk for MeHg contamination](#). As primary consumers whose body Hg concentration is the direct reflection of environmental
291 exposure rather than prey-derived transfer, soil-feeding termites are potentially suitable candidates as bio-indicators of mercury pollution [in soils of neotropical](#)
292 [rainforest ecosystems. This study was performed in chronically contaminated sites where termites likely tolerate the pollutant. Given the potential risk of MeHg](#)
293 [accumulation by termites on their predators, this study deserves to be extended to newly contaminated environments such as sites around artisanal and small-scale](#)
294 [gold mining \(ASGM\) that are extended worldwide including in tropical areas \(UN Environment, 2019; Gerson et al. 2018\), where termites are amongst the most](#)
295 [representative faunal groups. If the survival of termites and their mercury-methylation capacity as shown in this study are not affected when new mercury](#)
296 [contaminations occur, their use as bio-indicators Hg pollution could be extended to various tropical and subtropical ecosystems.](#)

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300 **Figures and tables**

301 **Figure 1.** Concentrations of total mercury (A), mobile mercury (B) and percentage of mobile mercury (relative to total Hg, C) in nests of the soil-feeding termite
302 *S. minutus* at Crique Combat and the nearby soil (0-10 cm depth). The concentrations are given for dry weights. The non-parametric Kolmogorov-Smirnov test
303 was performed separately for each mercury fraction. Different bold letters indicate significantly different values between nests and the nearby soils ($p < 0.05$).

304 **Figure 2.** Linear regressions between concentrations of total mercury in the nests and in the nearby soils (A), between total mercury and mobile mercury in the
305 nests and in the nearby soils (B), between total mercury and the percentage of mobile mercury (C), and between mobile mercury and the percentage of mobile
306 mercury (D) in all samples. The measurements are for *S. minutus* at Crique Combat. Shaded areas correspond to the 95% confidence interval; R is the Spearman
307 coefficient, p the p-value. The concentrations are given for dry weights.

308 **Table 1.** MeHg concentrations in the whole bodies of workers and nests for two soil-feeding termites, *S. minutus* and *S. holmgreni* at Crique Combat, in
309 comparison with the nearby soils. MeHg concentrations are given for dry weights and the percentage of MeHg relative to total mercury (THg) was calculated for
310 *S. minutus* nests and the nearby soils. The MeHg bioconcentration factors for the termite bodies were calculated relative to the nearby soils (BCF_{soil}) and the nests
311 (BCF_{nest}). One-way ANOVA was performed separately for each termite species and values with different bold letters differ at p <0.05.

312 **Table 2.** Distribution of MeHg in termite bodies between the whole gut and the gut-free carcass of *S. minutus* at Petit Saut, in comparison with the concentrations
313 in the nests and nearby soils. The concentrations of MeHg are given for dry weights and <DL indicates that the MeHg concentration in the soil was below the
314 detection limit of the analytical instrument. Consequently, BCFs were calculated only relative to nests. Values with different bold letters differ significantly
315 according to the one-way ANOVA at p<0.05.

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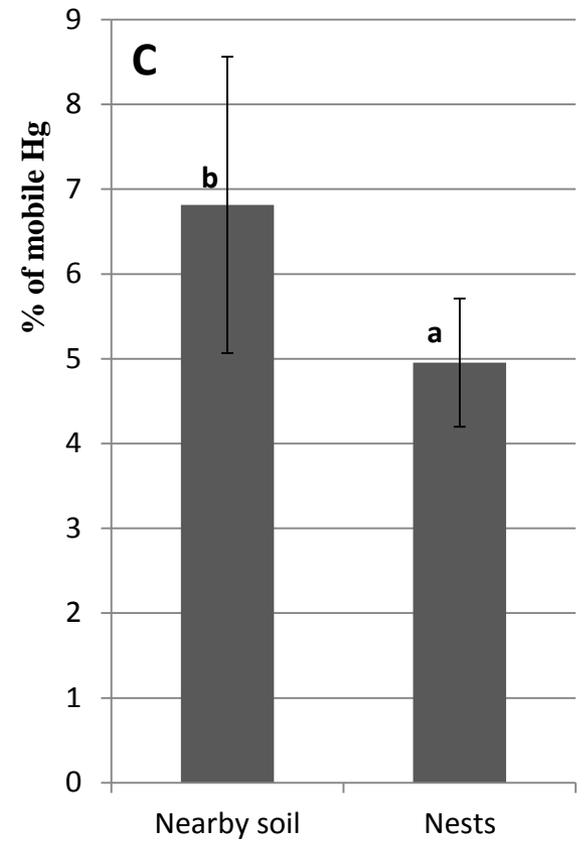
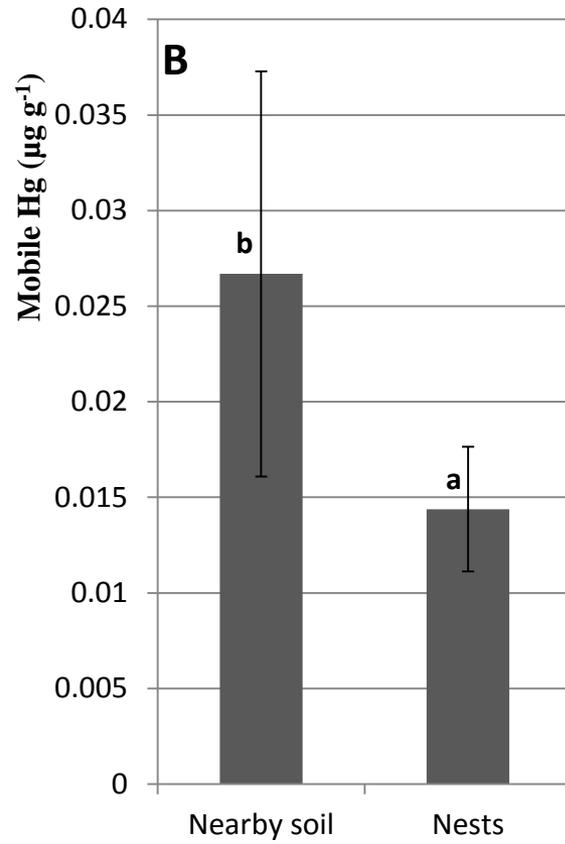
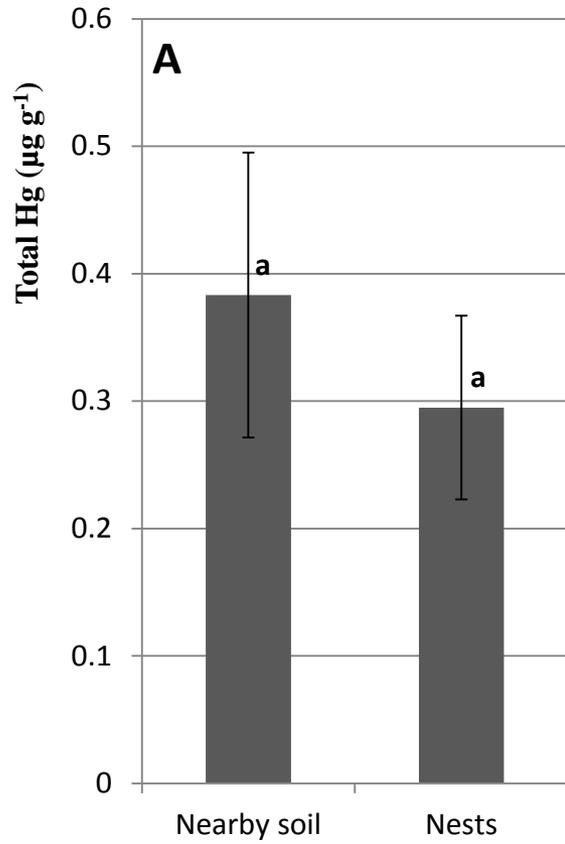
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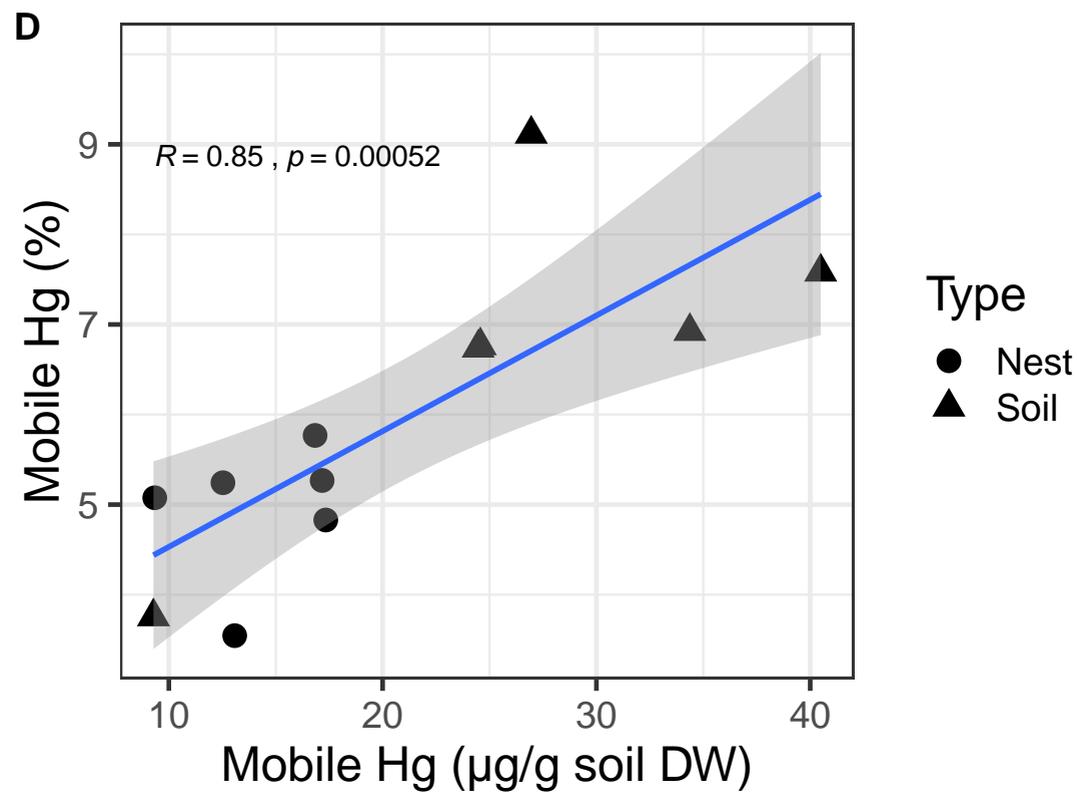
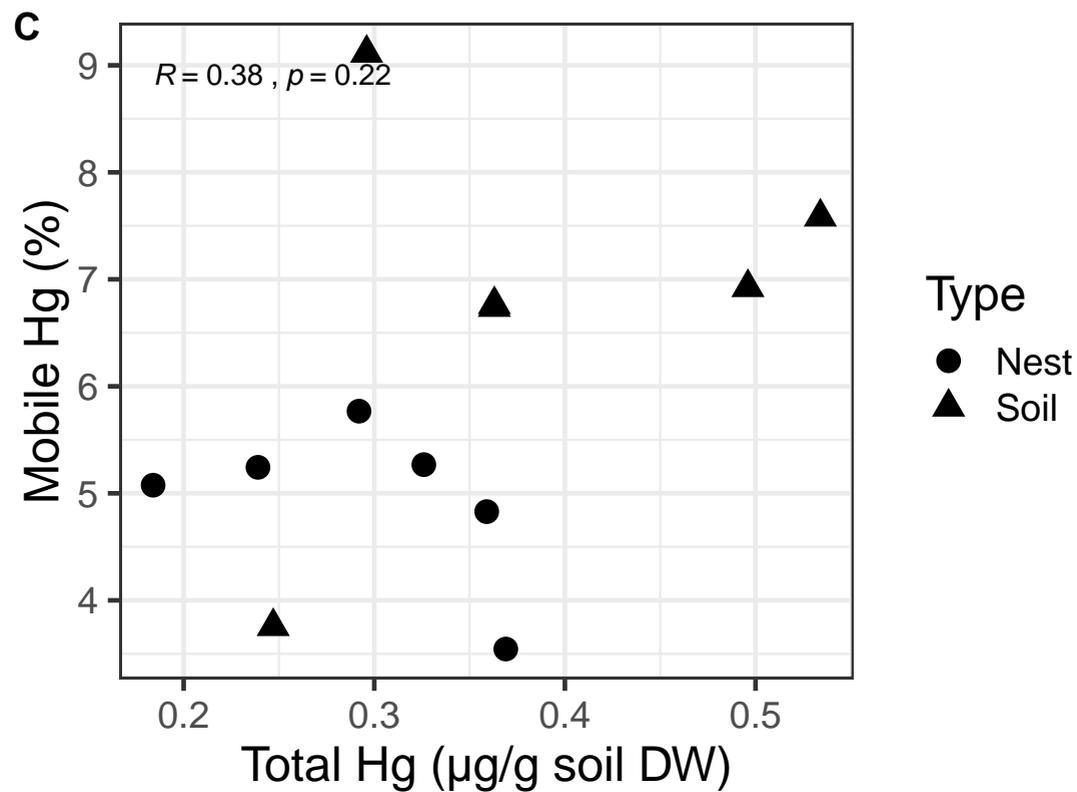
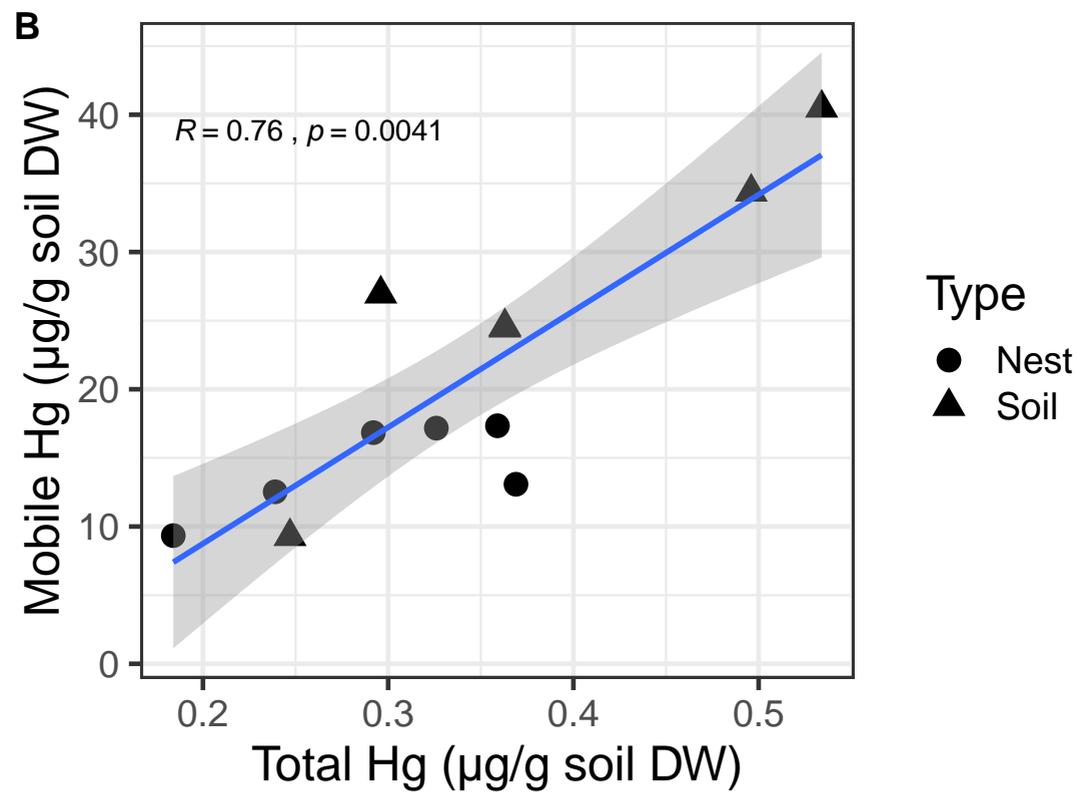
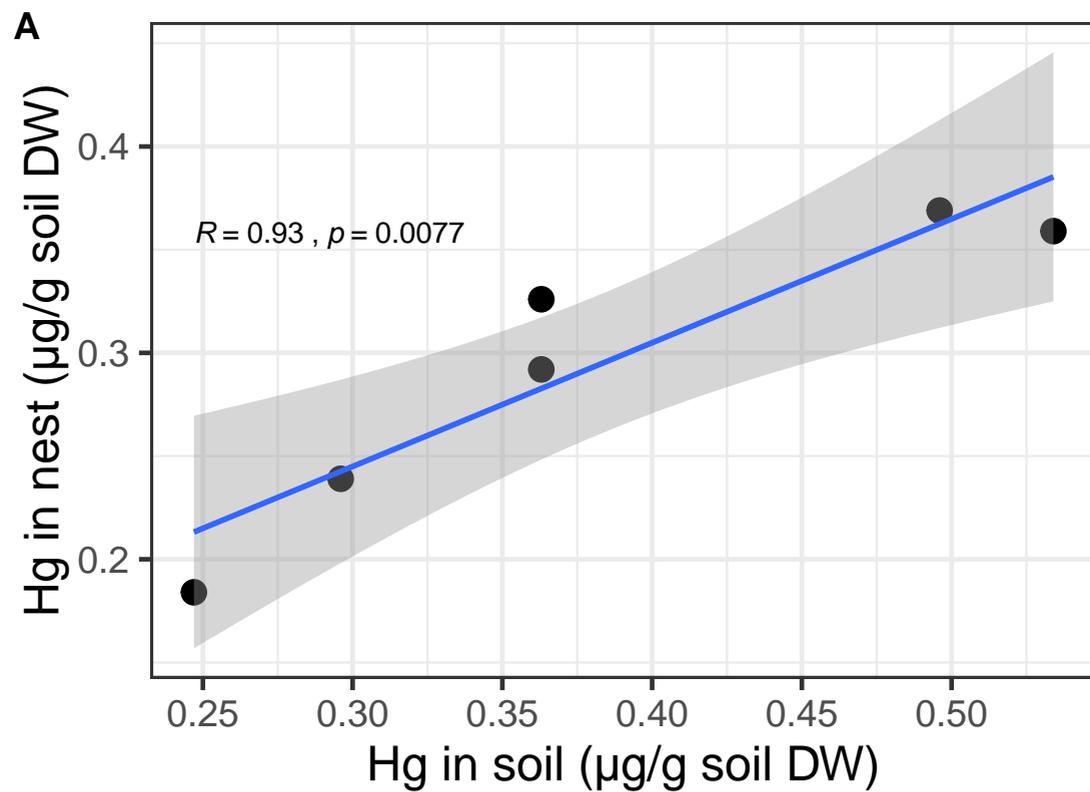
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	<i>S. minutus</i>			<i>S. holmgreni</i>	
	MeHg (ng g ⁻¹) (Mean ± SD)	Range (ng g ⁻¹)	% MeHg (% of THg)	MeHg (ng g ⁻¹) (Mean ± SD)	Range (ng g ⁻¹)
Nearby soils	0.66 ± 0.30 A	0.35 - 0.96	0.18 ± 0.10	0.48 ± 0.27 A	0.18 - 0.70
Nests	11.21 ± 6.70 B	5.93 - 22.78	4.73 ± 4.42	1.30 ± 0.32 A	0.94 - 1.67
Termite bodies	38.45 ± 28.83 C	15.29 - 94.47	---	38.01 ± 34.25 B	9.64 - 83.42
BCF _{soil}	57.84 ± 25.85	34.37 - 106.12		178.91 ± 234.73	23.33 - 448.91
BCF _{nest}	3.76 ± 1.61	1.87 - 5.56		33.64 ± 31.68	6.75 - 71.24

	MeHg (ng.g ⁻¹)	BCF (relative to nest)
Nearby soil	< DL	
Nest	4.22 ± 4.06 a	---
Termite carcass	683.33 ± 473.98 b	191.37 ± 53.59
Termite gut	221.13 ± 175.66 ab	58.14 ± 18.35

