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Validation and extension of the Tea Bag Index to collect decomposition data from termite-rich ecosystems

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ABSTRACT

The Tea Bag Index (TBI) is a standardised and cheap method to quantify microbial-driven decomposition by measuring the mass loss of tea within tea bags. Termites are known to damage the bags to access the content, rendering the method less suitable for termite-rich ecosystems. Extension of the TBI to accommodate and incorporate the influence of termites would broaden its applicability to include termite-rich ecosystems, such as tropical forests.

We extended the original TBI by applying physical and chemical termite-exclusion methods. Tea mass loss and the proportion of tea bags detected by termites in the original TBI were also recorded to infer the role of termites in litter decomposition. TBI estimates derived from the original and extended TBI were compared, benchmarked against global estimates, and validated with time-series mass loss data.

Using the original TBI, we found that termites damaged up to 80 % of tea bags and consumed the recalcitrant fraction of tea in several of them, leaving only 20 % of tea bags from which TBI estimates could be retrieved. The physical termite-exclusion treatment completely eliminated termite-infringement, thus preserving the full sample size for estimating TBI parameters. The chemical termite-exclusion treatment also successfully excluded termites, but potentially inhibited microbial decomposition and made TBI estimates unreliable. In the absence of termite-infringement, both the TBI estimates and time-series analysis revealed a low decomposition rate compared to other measurements in tropical and temperate regions.

We propose an extended TBI, in which the physical termite-exclusion treatment is used to preserve the retrieval rate of TBI parameters and reliably measure microbial-driven decomposition, while the original TBI is used to incorporate the contribution of termites in driving litter mass loss. By characterising both termite- and microbial-driven decomposition, the extended TBI will provide a comprehensive understanding of decomposition and its drivers in termite-rich ecosystems, and permit global comparisons.

1. Introduction

The decomposition of plant litter is a vital ecosystem process, forming a major pathway for the cycling of carbon and nutrients (Swift et al., 1979; Vitousek, 1982). The rate of decomposition is determined by climate, litter chemistry, and the decomposer community (Coûteaux et al., 1995). It regulates the availability of carbon and nutrients to plants (Cornwell and Weedon, 2014) and also governs the flux of carbon between the biosphere and atmosphere (Schimel, 1995; Aerts, 1997). Robust measurements of decomposition rates are thus essential for parameterising models of the global carbon cycle (Hättenschwiler et al., 2011). It is also crucial in providing a mechanistic understanding of the determinants of decomposition (Cornwell and Weedon, 2014). Consequently, more than 1000 studies have measured rates of decomposition (Prescott, 2005), employing a wide range of methods that make comparisons difficult.

The litterbag method (Wider and Lang, 1982) is commonly used to estimate decomposition; however, methodological diversity hampers comparison between studies. The method involves tracking the mass loss of plant litter contained within a mesh bag over time (Cornwell and

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Weedon, 2014). Comparison between decomposition studies using natural leaf litter is difficult because of the high inter- and intra-species variability in leaf chemistry (Aerts, 1997; LeRoy et al., 2007) and the strong influence of litter chemistry on decomposition rates (Parton et al., 2007). Cellulose filters are commonly used instead, but they are unrepresentative of the chemical composition of natural leaf litter (Fritz et al., 2011). The litterbag mesh size used also varies across studies (Kampichler and Bruckner, 2009), effectively filtering different size-classes of decomposer fauna (Bradford et al., 2002) and thus introducing another source of variance.

To improve comparability between studies, and to provide an accessible protocol for citizen scientists, Keuskamp et al., (2013) developed the Tea Bag Index (TBI). The TBI involves burying two widely-available types of tea bags (green and rooibos Lipton tea) for 90 days and then measuring mass loss. In contrast to the litterbag method, the contrasting decomposabilities of the two tea types permits concurrent estimation of both decomposition rate and stabilisation factor from a single time measurement. The lower variability in the chemical composition of tea also permits replicability across systems and hence enable other decomposition determinants, such as site-specific micro-environment and decomposer diversity, to be assessed. There has been an increasing trend to engage citizen scientists in ecology (Silvertown, 2009), allowing data to be collected across greater spatiotemporal scales (Dickinson et al., 2010). Combined with its ease of use and low cost, the TBI has served as a citizen science platform to collect global decomposition data.

The TBI is designed primarily to investigate microbial-driven decomposition, and the 0.25 mm tea bag mesh typically excludes many (though not all) mesofauna and macrofauna decomposers. Meso- and macrofauna are typically omitted in decomposition studies (Gessner et al., 2010; Hättenschwiler et al., 2011) as microbial decomposition dominates on a global scale (Swift et al., 1979), especially in temperate and boreal ecosystems (Hättenschwiler et al., 2005). However, in the humid tropics, faunal-driven decomposition is not insignificant (Gonzalez and Seastedt, 2001; Wall et al., 2008; Powers et al., 2009).

One of the most important macrofaunal decomposers in lowland tropical forests are termites (Eggleton and Tayasu, 2001; Bignell, 2006), which can form up to 95 % of the soil insect biomass (Watt et al., 1997). Given their large biomass and efficient foraging (Traniello et al., 2000), and their association with symbiotic gut microbiota that digest lignocellulose (Bignell, 2000; Brune and Ohkuma, 2011), termites process large quantities of plant litter (Jouquet et al., 2011) and contribute significantly to decomposition and carbon mineralisation (Lavelle et al., 1993; Schuurman, 2005; Jouquet et al., 2011). Furthermore, termites from the Macrotermitinae subfamily, which are found in tropical Africa and Asia, drive additional carbon mineralisation in plant litter via their obligate exosymbiosis with a fungal partner (Wood and Thomas, 1989). Overall, in tropical forests, termites are responsible for mineralising up to 11 % of the annual above-ground litterfall (Yamada et al., 2005) (Table S1).

While the TBI was designed to omit macrofauna, termites can chew through tea bag mesh, which presents a challenge for its use in the tropics. Indeed, termites have been documented chewing through plastics and thin pieces of lead (Gay and Calaby, 1970). Further, termites can decompose recalcitrant organic compounds even over short incubation periods (Lavelle et al., 1997), which prevents the estimation of TBI parameters in accordance with its underlying assumptions of negligible mass loss from the recalcitrant fraction over short field-incubations, and of a predominantly microbial-driven decomposition. Consequently, using TBI in the tropics would necessitate discarding termite-damaged bags, which is a loss of both sampling efficiency and the opportunity to learn more about termites' crucial role in driving litter mass loss and mineralisation (Donovan et al., 2001).

Therefore, in this paper, modifications are made to the original TBI to permit the reliable estimation of TBI parameters in termite-rich ecosystems, while also incorporating the functional contribution of termites in litter mass loss and decomposition. These modifications will be most pertinent in the humid tropics, given that the abundance and diversity of termites increase and peak towards the equator (Eggleton et al., 1994; Eggleton, 2000). For ecosystems in the higher latitudes, such as temperate and boreal forests, the original TBI will suffice, since termites are generally absent beyond 51° north or south of the equator (Eggleton, 1994, 2000).

Specifically, we have four study objectives:

1.) Quantify the contribution of termites in driving litter mass loss and determine their impact on the TBI in a termite-rich tropical forest.
2.) Remove the impact of termites on the TBI by implementing two termite-exclusion treatments, and comparing their effects on TBI estimates.
3.) Propose an extended TBI to obtain reliable TBI estimates in termite-rich ecosystems and to incorporate termite-driven mass loss. The proposed extension will explicitly cater to the differences in expertise and resource-availability between professional researchers and citizen scientists.
4.) Determine the reliability of TBI estimates obtained in our study, by benchmarking against global TBI estimates, and by assessing the ability of our TBI estimates to predict temporal mass loss patterns and decomposition rates that are congruent with time-series data.

2. Materials and methods

2.1. Study sites

This study was conducted in Singapore, an equatorial island-state (Fig. 1). The climate is aseasonal, with an average mean daily temperature of 27.0 °C and an annual rainfall of 2331 mm, with no months receiving less than 100 mm of rain on average (National Environment Agency, 2016). Lowland Dipterocarp forests historically covered most of Singapore's interior (Wong, 1987). Large-scale deforestation reduced the coverage of primary forests to just 0.2 % of Singapore's land area (Corlett, 1992), forming isolated remnant patches surrounded by old secondary forests. These patches of primary forest remain vital refugia for biodiversity (Turner and Corlett, 1996), including functionally important soil invertebrates such as termites (Foo, 2013).

We established a study site in each of the two largest primary forest fragments. These sites are located in forest reserves – one in Bukit Timah Nature Reserve (BT) (1°21′17.8″N, 103°46′26.0″E) and the other in Central Catchment Nature Reserve (CC) (1°21′6.3″N, 103°49′22.6″E) (Fig. 1). The soil types of these primary forests are oxisols and ultisols overlying granite, and are highly acidic (pH 3.5–4.2) and infertile, with very low concentrations of nitrogen and phosphorus (Grubb et al., 1994).

Within each site, the microclimate of the forest understorey was characterised (Table 1). Air temperature and humidity were recorded using data loggers (iButton DS1923, Dallas Semiconductor, TX, USA) placed in radiation shield housings elevated approximately 90 cm above the forest floor, while soil temperature and soil volumetric water content were obtained using sensors connected to a HOBO Micro Station data logger (Onset Computer Corporation, MA, USA).

2.2. Termite survey

Termites were surveyed in each site along a transect 100 m long and 2 m wide, following a transect sampling protocol designed for tropical forests by Jones and Eggleton (2000). All termite species were identified to species or morphospecies. The relative occurrence of each termite species across sampling quadrats was used as a proxy for relative abundance. Twenty-eight and 27 termite species were recorded in the BT and CC sites respectively (Table S2), representing the highest richness and relative abundance of termites recorded in Singapore (Foo, 2013). By including the fullest diversity of termite functional groups, a

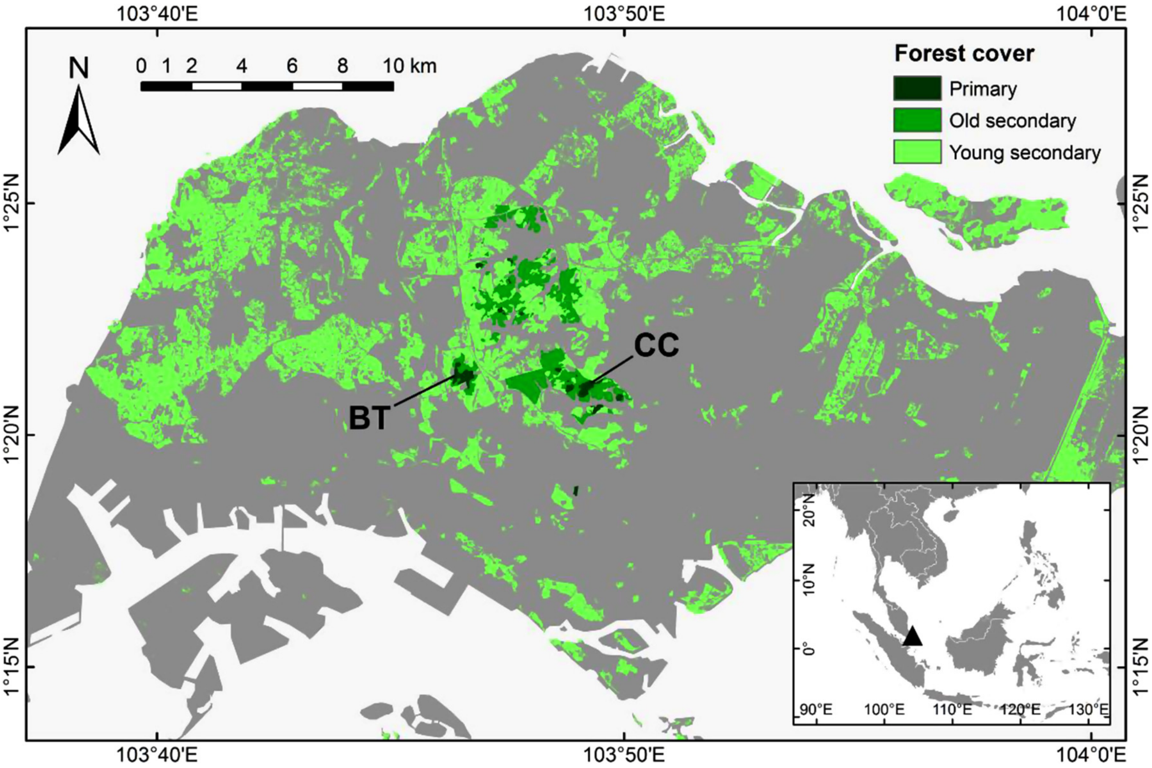

Fig. 1. Location of study sites BT (Bukit Timah Nature Reserve) and CC (Central Catchment Nature Reserve) in Singapore. Inset shows location of Singapore (triangle symbol) within Southeast Asia.

better characterisation of their functional role in litter mass loss and decomposition can be inferred.

2.3. Study design and termite-exclusion treatments

We tested the original TBI (see Tea Bag Index (2016) for stepwise protocol) by deploying Lipton green tea (EAN: 87 22700 05552 5) and Rooibos tea (EAN: 87 22700 18843 8) bags without any modifications in the control group, following Keuskamp et al., (2013). We also augmented the original TBI by incorporating termite-exclusion methods in two treatment groups, where termites were excluded from tea bags using either a physical or chemical barrier.

2.3.1. Physical termite-exclusion

Termites were excluded from tea bags by a wire cloth barrier made of high-grade corrosion-resistant stainless steel (Grade 316, Wire Mesh Industries Pte Ltd Singapore). The wire cloth has a wire diameter of 0.22 mm and a mesh aperture of 0.29 mm. This aperture size was chosen to ensure the exclusion of termites with high confidence, while still permitting the entry of microfauna, microbial decomposers, and most mesofauna. This mesh aperture closely matches the tea bag nylon mesh size of 0.25 mm. Also, studies have reported the effectiveness of a commercial termite barrier – TERMI-MESH® (TERMI-MESH Australia Pty Ltd), which uses a similar grade of stainless steel but has an even larger aperture size of 0.66 mm by 0.45 mm, against various species of subterranean termites (Lenz and Runko, 1994; Grace et al., 1996). Individual tea bags were enveloped in separate packets made from the wire cloth (Fig. S2). Open sides of each packet were folded thrice, with each fold hammered down to ensure a tight seal.

2.3.2. Chemical termite-exclusion

Given the high costs of physical termite barriers (Su and Scheffrahn, 1998), we also tested a chemical termite-exclusion method which is cheaper and more time-efficient. We used a commercial pyrethroid termiticide – bifenthrin, which is commonly used to protect building structures from subterranean termites (Su and Scheffrahn, 1998). Bifenthrin works primarily by repelling termites instead of killing them, and is effective at very low concentrations (Su and Scheffrahn, 1990; Yeoh and Lee, 2007; Sharma et al., 2009). The repellence of Bifenthrin persists for periods well exceeding our study duration (Sharma et al., 2009; Peterson, 2012).

We used a commercial bifenthrin formulation (Newthrin 9% S.C, New Eastern (1971) Pte Ltd, Singapore) and diluted it to a manufacturer's recommended concentration of 750 ppm (of bifenthrin), which should provide adequate termite-repellence, given that 100 ppm was already effective in repelling subterranean termites (Su and Scheffrahn, 1990; Yeoh and Lee, 2007). We soaked tea bags in the bifenthrin solution for one hour, after which they were dried in a drying oven. Due to bifenthrin's strong binding with organic matter (Baskaran et al., 1999; Peterson, 2012), it will not leach into water sources easily

Table 1
Microclimatic parameters of the forest understorey in each study site recorded using data loggers over a total of 108 sampling days.

Study site	Mean air temperature ± SE (°C)			Mean daily relative humidity ± SE (%)	Mean daily soil volumetric water content ± SE ($m^3\ m^{-3}$)	Mean daily soil temperature ± SE (°C)
	Daily mean	Daily maximum	Daily minimum			
BT	26.2 ± 0.1	28.6 ± 0.1	24.5 ± 0.1	91.1 ± 0.4	0.245 ± 0.002	25.77 ± 0.04
CC	26.2 ± 0.1	28.9 ± 0.1	24.4 ± 0.1	93.6 ± 0.4	0.125 ± 0.002	26.24 ± 0.04

(Sharma et al., 2009). Also, being a pyrethroid class of termiticide, bifenthrin poses very low risks to the environment and human health (Yeoh and Lee, 2007).

2.3.3. Field-deployment

Forty tea bags (20 Rooibos tea and 20 green tea) were used in each treatment and control group, for a total of 120 bags. For each tea bag, initial mass of the *tea* litter was obtained after subtracting the average mass of an empty nylon *tea bag* (using 20 tea bags selected at random). Termiticide-treated tea bags were weighed prior to and after soaking in termiticide solution. The post-soaking tea mass was used as the initial mass, while the pre-soaking tea mass served as a correction factor to adjust the hydrolysable fraction which is altered after soaking (Eq. 1). For Rooibos tea, the average mass loss due to soaking was 0.120 $g\ g^{-1}$ while that for green tea was 0.291 $g\ g^{-1}$.

Within each study site, tea bags were deployed at five locations distanced 30 m apart in a grid layout. Rooibos and green tea bags were deployed in pairs, following Keuskamp et al., (2013). The leaf litter layer was cleared and tea bags were buried just beneath the soil surface in 4 cm deep holes. At this burial depth, tea bags will be accessible by both soil- and litter-feeding termites. All tea bags were secured to a stake to prevent loss and to facilitate retrieval. Termiticide-treated tea bags in the chemical termite-exclusion treatment were distanced at least 3 m from other tea bags to avoid potential secondary effects.

Tea bags were incubated in the field for 90 days from 25 February till 26 May 2015, after which they were retrieved and dried to constant mass at 65 °C. We recorded evidence of perforations on the tea bag's nylon mesh. We considered tea bags to be detected by termites if perforations ≥ 3 mm are present (Fig. S1). We have a high degree of confidence that these larger holes were largely, if not fully, attributable to the feeding activity of termites. Small perforations < 3 mm were taken to be caused by mechanical damages.

After drying, tea bags were carefully opened and foreign debris such as roots and soil particles were removed. Tea mass loss per tea bag was obtained and expressed in relative fractional terms. The mass loss of termite-undetected tea bags was attributed primarily to microbial decomposition, while the additional mass loss in termite-detected tea bags was attributed to termites. To test for the presence of a termite-detection and treatment effect, we analysed mass losses with Kruskal-Wallis nonparametric analysis of variance, using the kruskal.test function in R (R Core Team, 2016). A post-hoc two-tailed Dunn's test (Dunn, 1964), with p-values corrected for multiple comparisons using a Benjamini-Hochberg adjustment (Benjamini and Hochberg, 1995), was then performed to compare treatment pairs, using the dunnTest function in the package 'FSA' (Ogle, 2016).

2.4. Calculation of TBI

TBI parameters were calculated following equations by Keuskamp et al., (2013). An exception was made for the chemical termite-exclusion treatment, where we corrected for the change in hydrolysable fraction of both Rooibos and green tea by:

$$H_{post} = 1 - \frac{W_{pre}}{W_{post}}(1 - H_{pre}) \tag{1}$$

where H_{post} is the corrected hydrolysable fraction post termiticide-soaking, H_{pre} is the original hydrolysable fraction by Keuskamp et al., (2013), W_{pre} is the tea mass before termiticide-soaking, and W_{post} is the tea mass after termiticide-soaking. This correction assumes that all mass loss from termiticide-soaking originated from the hydrolysable fraction.

For the physical termite-exclusion treatment, we recorded negative values of S (stabilisation factor), resulting from mass losses of green tea that exceeded H_g (hydrolysable fraction of green tea) (Table 2). The numerical values of $S < 0$ were respected in the subsequent derivations of a_r (estimated hydrolysable fraction of Rooibos tea). In doing so, we acknowledged the uncertainty in the measurement of H_g by Keuskamp et al., (2013), which had a standard deviation of 0.023. All termite-undetected green tea bags in our study had mass losses that fall within H_g after taking into account its measurement uncertainty.

TBI parameters were estimated following either a paired or unpaired approach, and compared between the control and treatment groups. In the paired approach, each tea bag pair served as a unit of replication. TBI parameters were estimated on a pairwise basis, and was hence dependent on the mass loss data from both tea bags in a pair. Given the TBI's inherent assumption of a negligible mass loss of the recalcitrant fraction for short-term field incubations (Keuskamp et al., 2013), all tea bag pairs where at least one of the tea bag is termite-detected had to be omitted from TBI calculations.

In the unpaired approach, individual tea bags were treated as replicates instead. Termite-detected *tea bags*, rather than termite-detected *tea bag pairs*, were omitted from the dataset. TBI parameters were then estimated on a study site or plot level. Individual estimates of S were pooled from all termite-undetected green tea bags to derive a mean site-level estimate of S, and subsequently a mean site-level estimate of a_r. Thereafter, k was estimated from each termite-undetected Rooibos tea bag using the site-level mean a_r.

2.5. Observed temporal mass loss

To determine the ability of TBI estimates to predict actual decomposition rates and temporal mass loss patterns resulting from microbial decomposition, we conducted a time-series study concurrently in the same sites, using only Rooibos tea bags. Fifty tea bags were deployed in each site, and 10 tea bags were recovered from each site at each incubation period (7, 14, 28, 56, and 112 days). Mass loss of termite-undetected tea bags was fitted to the exponential function:

$$W_r(t) = a_r e^{-kt} + (1 - a_r) \tag{2}$$

using a non-linear least square model (Gauss-Newton algorithm), where $W_r(t)$ is the relative mass of Rooibos tea remaining after incubation time t (days), a_r is the labile fraction of Rooibos tea, $(1 - a_r)$ is its recalcitrant fraction, and k is its decomposition rate (day^{-1}).

3. Results

3.1. Influence of termites on the TBI and on mass loss

Termites had a sizeable effect on the TBI, as evidenced by the high proportion of termite-detected tea bags – 65 % of Rooibos tea bags and 60 % of green tea bags in the control group were detected. On a paired tea bag basis, 80 % of all tea bag pairs in the control group had at least one tea bag detected by termites, with Rooibos and green tea bags exhibiting comparable levels of detectability (Table 3).

The substantial role of termites in driving litter mass loss was demonstrated here. Tea bags detected by termites had greater mass losses compared to undetected tea bags, although this pattern was confined to Rooibos tea. In the control group, termite-detected Rooibos tea bags had an average mass loss of 0.485 $g\ g^{-1}$, compared to 0.333 $g\ g^{-1}$ for termite-undetected tea bags (Fig. 2). However, the large heterogeneity in mass loss of termite-detected tea bags (Fig. 1 inset) meant that the increase was not statistically significant (Dunn's pairwise comparison, $Z = -0.83$, $p = 0.487$). The strong influence of termites was not mirrored amongst green tea bags. The mass loss of termite-detected and undetected green tea bags averaged 0.833 $g\ g^{-1}$ and 0.795 $g\ g^{-1}$ respectively (Fig. 2), and this small difference was not statistically significant (Dunn's pairwise comparison, $Z = -1.50$, $p = 0.200$).

The potential of termites to consume the recalcitrant fraction of tea was also demonstrated in our study. When TBI was calculated using the paired approach, we observed termite-detected Rooibos tea bags with mass losses greater than a_r and even H_r, with two tea bags exhibiting mass losses that approached 1.00 $g\ g^{-1}$, providing clear evidence of

Table 2

Estimated values of TBI parameters (mean ± SE) for each study site across different treatment groups, where S = stabilisation factor, a_r = decomposable fraction of Rooibos tea, and k = decomposition rate. The sample sizes (number of termite-undetected tea bags/pairs) available for each treatment and study site are shown. TBI calculations followed either the paired or unpaired approach (see methods). Values in square brackets indicate the number of S estimates < 0. Values in parentheses indicate the number of k estimates removed, due to the presence of Rooibos tea bags with mass loss exceeding estimated a_r and resulting in mathematical invalidity.

		Control (termite-undetected tea bags)		Physical termite-exclusion treatment		Chemical termite-exclusion treatment	
Study site	Tea Bag Index estimates	Paired	Unpaired	Paired	Unpaired	Paired	Unpaired
BT	S	0.085 ± 0.024	0.068 ± 0.018	0.016 ± 0.011 [4]	0.016 ± 0.011 [4]	0.355 ± 0.018	0.355 ± 0.018
	a_r (g g^{-1})	0.505 ± 0.013	0.515 ± 0.080	0.543 ± 0.006	0.543 ± 0.064	0.314 ± 0.009	0.314 ± 0.067
	k (day^{-1})	0.012 ± 0.002	0.012 ± 0.001	0.0098 ± 0.001	0.0098 ± 0.001	0.015 ± 0.004	0.012 ± 0.003 (1)
	Termite-undetected tea bags (n)	4 pairs	6 Rooibos 6 green	10 pairs	10 Rooibos 10 green	9 pairs	10 Rooibos 9 green
CC	S	–	0.021 ± 0.011	0.025 ± 0.011 [3]	0.025 ± 0.011 [3]	0.222 ± 0.037	0.222 ± 0.037
	a_r (g g^{-1})	–	0.540 ± 0.064	0.538 ± 0.006	0.538 ± 0.064	0.375 ± 0.018	0.375 ± 0.095
	k (day^{-1})	–	0.012	0.011 ± 0.001	0.011 ± 0.001	0.012 ± 0.002 (1)	0.011 ± 0.002 (1)
	Termite-undetected tea bags (n)	0 pair	1 Rooibos 2 green	10 pairs	10 Rooibos 10 green	10 pairs	10 Rooibos 10 green

mass loss within the recalcitrant fraction (Fig. 3). Also, five tea bag pairs with termite-detected green tea bags had mass losses exceeding H_g (Fig. 3). It is impossible to derive estimates of k for these tea bag pairs as the labile fraction can no longer be determined, and since its decomposition rate is no longer proportional to its mass loss. Tea bags that had mass loss from the non-hydrolysable (recalcitrant) fraction always corresponded with detection by termites (Fig. 3). Conversely, all tea bag pairs where both tea bags were not detected by termites fulfilled the assumptions of TBI (see caption in Fig. 3).

The omission of termite-detected tea bags represented a substantial reduction in the TBI dataset. Furthermore, the complete absence of termite-undetected tea bag pairs from one of the study site (CC) resulted in the inability to estimate TBI parameters (Table 2). For the BT site, even after excluding termite-detected tea bag pairs, mass losses from green tea bags approached the entire H_g. Correspondingly, low estimates of S, averaging 0.0845, were obtained. This resulted in estimates of a_r that almost approached H_r. However, the observed mass loss for Rooibos tea was considerably lower than its estimated a_r, hence a low k of 0.0115 day^{-1} was obtained (Table 2).

When TBI was calculated using the unpaired approach, a greater preservation of sample size was achieved, since not all tea bag pairs had both tea bags detected by termites. However, the sample size remained small, especially for the CC site (Table 2). By pooling all termite-undetected green tea bags in the CC site, we obtained a very low site-level S estimate of 0.0213. For the BT site, although the unpaired approach produced slight changes in the estimates of S and a_r compared to those obtained following the paired approach, the mean estimate of k remained very similar, at 0.0117 day^{-1} (Table 2). Furthermore, the unpaired approach generated a similar estimate of k for the CC site too, which averaged 0.0118 day^{-1} (Table 2). Nonetheless, the sample size for the CC study site is very small (n = 1 Rooibos tea bag), making any inter-site comparison of k estimates highly unreliable.

3.2. *Testing the effectiveness of physical and chemical termite-exclusion treatments*

Both physical and chemical termite-exclusion treatments excluded termites from tea bags effectively. The physical barrier completely eliminated termite detection, while the chemical barrier was nearly as effective, and prevented termites from attacking all but one green tea bag (Table 2).

The physical termite-exclusion treatment was also effective in reducing tea mass loss attributed to termites, at least for Rooibos tea, where mass loss was reduced to an average of 0.317 g g^{-1}, compared to the average of 0.485 g g^{-1} for termite-detected tea bags in the control group (Fig. 2). However, due to the large heterogeneity in mass loss of termite-detected tea bags, the reduction in mass loss was not statistically significant (Dunn's pairwise comparison, $Z = 1.60$, $p = 0.163$). For green tea, the physical termite-exclusion treatment had no statistically significant effect on mass loss when compared to the termite-detected tea bags in the control group (Fig. 2) (Dunn's pairwise comparison, $Z = 0.468$, $p = 0.640$). When comparing the mass loss between termite-undetected tea bags in the control group and tea bags in the physical termite-exclusion treatment, there were no statistical significance for both Rooibos (Dunn's pairwise comparison, $Z = 0.414$, $p = 0.679$) and green tea (Dunn's pairwise comparison, $Z = -1.23$, $p = 0.263$).

The chemical termite-exclusion treatment, albeit nearly matching the effectiveness of the physical termite-exclusion treatment in preventing termite-detection, caused a significant reduction in the amount of tea mass loss for both Rooibos (Dunn's pairwise comparison, $Z = 3.41$, $p = 1.92 \times 10^{-3}$) and green tea (Dunn's pairwise comparison, $Z = 5.57$, $p = 1.55 \times 10^{-7}$), when compared against the physical termite-exclusion treatment (Fig. 2). Therefore, the pattern of tea mass loss no longer mirrored that of termite-undetected tea bags in the control group.

Table 3

The probability of detection by termites amongst individual tea bags and tea bag pairs for the control and termite-exclusion treatment groups. Tea bag pairs were taken to be detected by termites when either tea bag, or both, were detected by termites. Within all termite-detected tea bag pairs, the pattern of detection in relation to tea type is also shown.

	Probability of detection by termites			Pattern of detection by tea type within termite-detected tea bag pairs (relative proportion)		
	Among individual tea bags		Among tea bag pairs			
Treatment group	Rooibos tea	Green tea		Only Rooibos tea bag	Only green tea bag	Both Rooibos and green tea bags
Control	0.65	0.60	0.80	0.25	0.1875	0.5625
Physical termite-exclusion	0	0	0	0	0	0
Chemical termite-exclusion	0	0.05	0.05	0	1	0

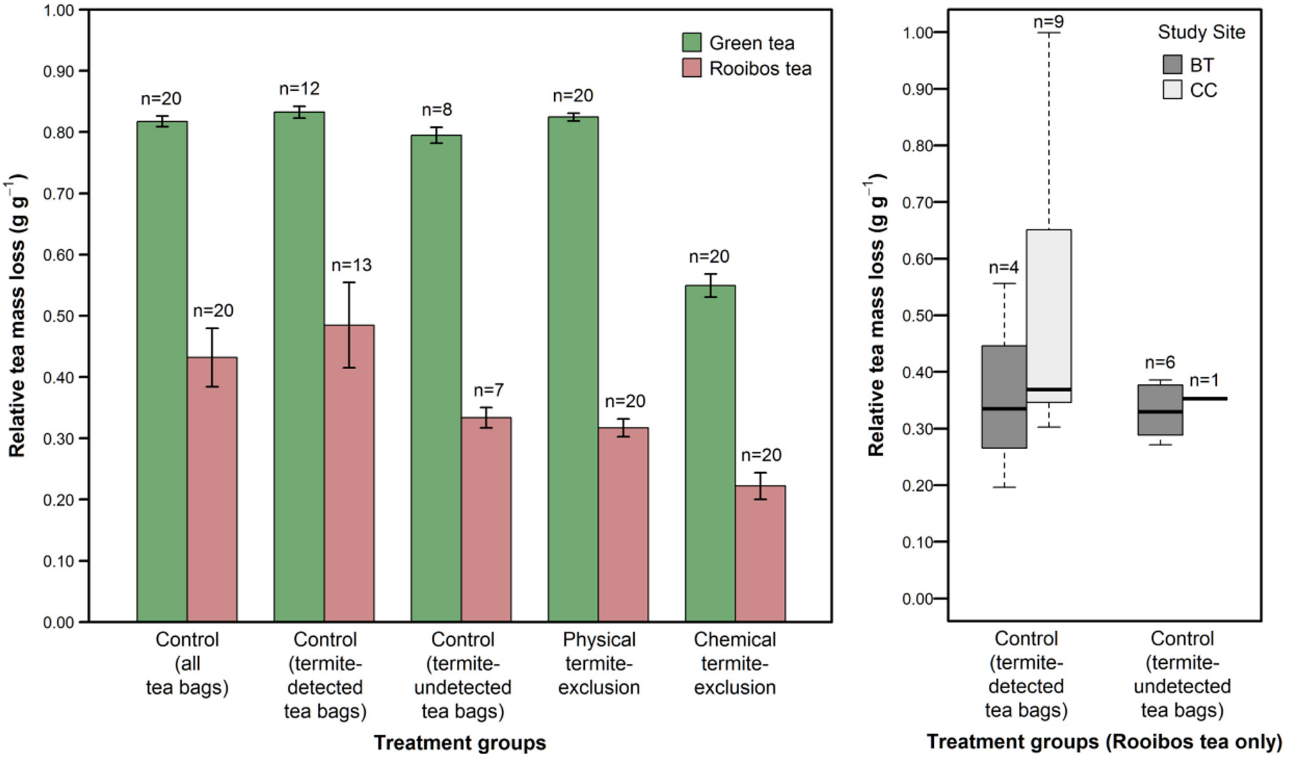

Fig. 2. Relative mass loss of green and Rooibos tea after a 90-day field incubation. Error bars represent standard error of the mean. Tea bags in the control group are further split into termite-detected and -undetected tea bags. Number of tea bags are labelled on top of each bar. In the inset, box plots show the mass loss of termite-detected and -undetected Rooibos tea bags in the control group per study site. Note: BT – Bukit Timah Nature Reserve; CC – Central Catchment Nature Reserve.

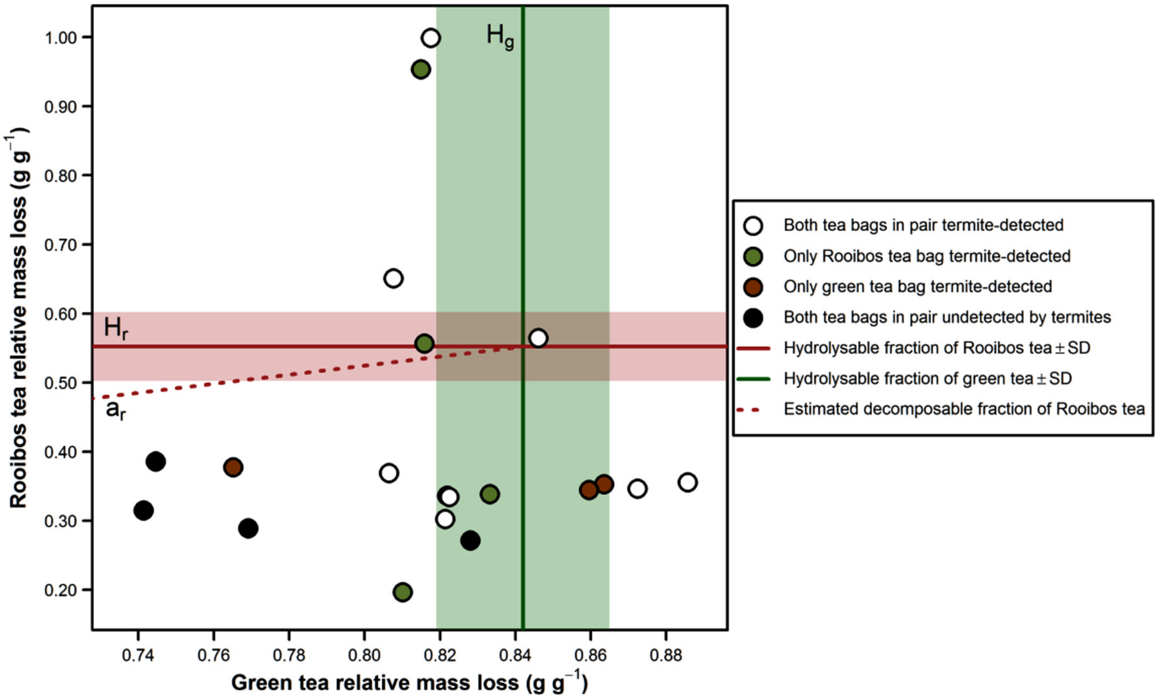

Fig. 3. Observed tea mass losses within the control group. Symbols indicate mass losses of individual tea bag pairs. Standard deviations in the hydrolysable fractions of the two tea types are represented by shaded regions. Calculations of TBI parameters in fulfilment of its assumptions will be impossible and/or problematic for tea bag pairs with mass losses exceeding defined thresholds, i.e. symbols above the dotted line and/or to the right of the vertical line. Here, we show that when using a paired approach, only tea bag pairs where both tea bags were undetected by termites always fulfilled TBI assumptions and hence can be used to estimate TBI reliably.

3.3. Calculations of TBI with termite-exclusion treatments applied

The physical termite-exclusion treatment enabled the full preservation of sample size used to estimate TBI parameters, since all tea bags were termite-undetected. For the BT site, estimates of k yielded by the paired and unpaired approach were almost identical, at 0.00981 day^{-1} and 0.00982 day^{-1} respectively (Table 2). Similarly, for the CC site, the estimates of k derived following the paired and unpaired approach were nearly identical, at 0.0105 day^{-1} and 0.0106 day^{-1} respectively (Table 2).

Estimates of S obtained from the physical termite-exclusion treatment differed substantially from those obtained from termite-undetected tea bags in the control group, despite the close match in mass loss. When following the paired approach for the BT site, the physical termite-exclusion treatment generated an S estimate of 0.0161, compared to the S estimate of 0.0845 generated from termite-undetected tea bags in the control group (Table 2). Moreover, a caveat lies in the markedly reduced sample size of termite-undetected tea bags in the control group, which may not permit a reliable comparison of TBI estimates with the physical termite-exclusion treatment. This is compounded by the inability to compare S estimates derived from the termite-undetected tea bags in the control group with those from the physical termite-exclusion treatment in the CC site, due to the complete absence of termite-undetected tea bag pairs in the control group.

The chemical termite-exclusion treatment produced vastly different estimates of S and a_r that had no parallels with both the physical termite-exclusion treatment and termite-undetected tea bags in the control group, despite its effectiveness in eliminating termite-detection. Even after correcting for the altered initial hydrolysable fractions, the large reduction in tea mass loss resulted in a pronounced increase in S by at least four times (Table 2). Consequently, estimates of a_r were reduced. Moreover, several Rooibos tea bags had mass loss that exceeded the threshold defined by a_r, resulting in mathematically undefined (log of negative number) estimates of k which had to be omitted. Also, the paired and unpaired approach produced slightly different k estimates (Table 2). Nonetheless, the estimates of k following both TBI calculation approaches were largely congruent with those obtained from termite-undetected tea bags in the control group and from the physical termite-exclusion treatment, in spite of the marked increases in estimates of S and decreases in estimates of a_r.

Overall, regardless of the termite-exclusion treatments or the approaches used to estimate TBI parameters, the response of k is greatly muted in comparison to that of S. Estimates of k fell within a narrow range of 0.0098 day^{-1} to 0.0148 day^{-1}. Taken together, our results point towards a low stabilisation of the labile fraction and a very low decomposition rate.

4. Discussion

4.1. Influence of termites on the TBI

The substantial consumption of tea, including its recalcitrant fraction by termites, was demonstrated here. The TBI's central assumption – that decomposition is predominantly limited to microbial decomposition of tea's labile fraction, was compromised amongst termite-detected tea bags. While this could be resolved by excluding termite-detected tea bags from further analysis, this would greatly diminish the sample size. By including termite-detected tea bags in analyses, the functional role of termites in driving litter mass loss, which is non-negligible in tropical forests, can also be estimated. Therefore, in this paper, we propose extensions to the TBI to facilitate its application in tropical forests.

4.2. Comparing physical and chemical termite-exclusion treatments on the TBI

By completely excluding termites, the physical termite-exclusion treatment allowed only the labile fraction of tea to be subjected to decomposition, primarily by microbes, hence fulfilling the TBI's assumptions. Moreover, the wire cloth used did not appear to alter the decomposition microenvironment within and around tea bags, given the similarities in mass loss between termite-undetected tea bags in the control group and tea bags in the physical termite-exclusion treatment. Furthermore, fungal hyphae and plant roots were observed to grow across the mesh aperture, further suggesting that microbial colonisation of the tea substrate was unhampered.

On the other hand, the application of the chemical termite-exclusion treatment introduced an inadvertent side-effect – the substantial leaching of labile components. Adjustments of the initial hydrolysable fractions were necessary, and this made the comparisons of TBI estimates with other studies difficult. In addition, the termiticide potentially inhibited microbial activity. Indeed, pyrethroid pesticides have been found to supress fungal growth and abundance, especially when applied at higher concentrations (Reddy and Natarajan, 1994; Nasim et al., 2005). Despite its low-cost and simplicity of application, we cannot recommend the chemical termite-exclusion method. Moreover, the application of toxic chemicals to a beverage product poses serious safety concerns, especially for citizen scientists.

4.3. Important functional role of termites in driving litter mass loss and decomposition remains neglected

Although the physical exclusion of termites allowed for an unimpeded characterisation of microbial decomposition that fulfilled TBI's assumptions, the important functional contribution of termites in driving litter mass loss, as demonstrated in the control group, remains overlooked. Indeed, by comparing termite-detected and undetected Rooibos tea bags within the control group, it can be inferred that consumption by termites resulted in a 46 % relative increase in mass loss. While not all litter mass ingested by termites will be eventually decomposed (*sensu* mineralised), there is evidence that termites are capable of mineralising 42 % of the carbon within consumed litter (Konate et al., 2003). On a stand-level in tropical forests, the estimates of mineralisation by termites vary widely (Table S1), with studies demonstrating that termites can mineralise as much as 10 % of the total annual litterfall (Yamada et al., 2005; Lopes de Gerenyu et al., 2015), hence strongly suggesting that termites represent a non-negligible contributor to carbon mineralisation in tropical forests.

The process of litter consumption by termites also indirectly promotes carbon mineralisation by microbial decomposers by increasing the surface area of litter available for microbial colonisation (Gessner et al., 2010; Jouquet et al., 2011). Furthermore, the faecal matter produced by termites is highly unstable and easily decomposed (Lavelle, 1997; Jungerius et al., 1999), thus further enhancing the completion of mineralisation of the original ingested litter. Henceforth, given that litter consumption by termites greatly facilitates and enhances carbon mineralisation (Lavelle et al., 1993), it is imperative that the TBI's design and protocol be extended to explicitly account for termites and incorporate termite-driven mass loss when deployed in termite-rich ecosystems.

While our study failed to show an increase in termite-driven mass loss for green tea, it does not imply a diminished role of termites in litter decomposition. The high proportion of labile compounds in green tea suggests that its mass loss was attributed mainly to microbial decomposition and leaching. Conversely, termites likely preferentially consumed Rooibos tea, which given its C:N of 42.9 ± 1.8, could represent a carbon-rich substrate accessible by termites, given their ability to decompose lignified material (Brune, 2014). On the other hand, the lower C:N of green tea (12.2 ± 0.1) potentially favoured microbial decomposition, which is enhanced by nitrogen-rich litter (Enriquez et al., 1993).

4.4. Proposed extensions to the TBI for application in termite-rich ecosystems

To enhance the applicability of TBI in termite-rich tropical forests, we propose two extensions: (1) the exclusion of termites to ensure a reliable characterisation of microbial decomposition and hence obtain robust TBI estimates, and (2) the incorporation of termites' contribution in driving tea mass loss, hence allowing their functional role in driving litter mass loss and decomposition to be inferred. The extended TBI will adopt separate protocols customised for professional researchers and citizen scientists (see Fig. S3).

To exclude termites, we recommend augmenting the TBI with the physical termite-exclusion treatment, given its demonstrated effectiveness and reliability over the chemical method. However, we propose that the physical termite-exclusion method be adopted primarily by professional researchers, since the difficulty and high costs involved in procuring high-grade wire cloth may deter citizen scientists. Moreover, the procedure involved is time- and labour-intensive, and thus impractical for citizen scientists. On the other hand, the investment of time and labour will be confined to the initial stages, since the stainless-steel mesh is non-biodegradable and can be reused for multiple studies. TBI parameters will then be estimated following the site-level unpaired tea bag approach (see methods section).

For citizen scientists, instead of implementing a termite-exclusion method, we recommend that they continue to deploy the original TBI (Fig. S3). However, citizen scientists will be required to check for the presence of termite-induced holes on tea bags. In anticipation that a large proportion of tea bags will be detected by termites and omitted from subsequent TBI calculations, we recommend that citizen scientists deploy at least 50 tea bag pairs per study site; preferably more if time and resources permit. To put into context, a deployment of 50 tea bag pairs should yield at least 5 Rooibos tea bags and 10 green tea bags undetected by termites after a 90-day incubation – a conservative estimate inferred from the CC site, which had a higher termite-detection probability than the BT site. In conjunction, we propose that the TBI calculations follow the unpaired approach. By treating each *tea bag*, rather than a *tea bag pair* as a unit of replication, the number of termite-detected replicates to be omitted from the calculation of TBI will be reduced, hence preserving sampling effort and the retrieval yield of TBI estimates.

To incorporate and infer termites' contribution in litter mass loss and decomposition, we propose that professional researchers supplement the physical termite-exclusion treatment with unmodified tea bag pairs as per the original TBI (Fig. S3). We recommend deploying at least 50 supplementary tea bag pairs per study site. For both professional researchers and citizen scientists, instead of discarding termite-detected tea bags, the mass loss of all tea bags should be recorded and presented (similar to the control group in Fig. 2). For termite-detected tea bags, it is essential to ensure the removal of soil transported into tea bags prior to weighing. Although termite-detected tea bags cannot contribute TBI estimates, their mass loss data, together with those of termite-undetected tea bags, can contribute valuable inferences on the relative contributions of termites and microbes in driving litter mass loss; the use of traditional litterbags renders it almost impossible to differentiate mass losses due to termites and microbial decomposers (Bignell and Eggleton, 2000). Moreover, the probability of tea bags detected by termites can also provide insights into the abundance of termites and their feeding-group composition, though this can only be tested with sufficient deployments of the extended TBI across tropical forests. Altogether, the output parameters of the extended TBI will consist of the estimates of k and S, microbial- and termite-driven mass loss, and the probability of tea bags detected by termites.

Furthermore, to account for the spatial heterogeneity in termite-driven decomposition, we recommend deploying the extended TBI at high spatial resolutions. Spatial heterogeneity was evident in our study, where despite the close proximity (< 6 km), the similarities in forest-type, termite richness, and termite abundance (Table S2), the two sites exhibited substantial differences in termite-detection probability and termite-driven mass loss (Fig. 2 inset). The spatial heterogeneity likely reflects site-specific factors, such as soil humus depth and forest disturbance levels, which shapes termite community assemblage and functional diversity (Davies et al., 2003). Nonetheless, we acknowledge that the low number of replicates and study sites used in our study hampers the identification of such linkages. By increasing the spatial resolution of future TBI deployments, linkages between termites' functional diversity and decomposition rates, which remain poorly characterised (Wall et al., 2008), can also be identified.

4.5. The need to validate the extended TBI in tropical forest ecosystems

The extended TBI should be field-tested in other tropical forests, particularly in forest-monitoring plots with well-characterised environmental properties, given that site-specific factors are important determinants of decomposition in the tropics (Powers et al., 2009). More importantly, there remains a paucity of TBI estimates from tropical forests, compared to temperate ecosystems. The widespread implementation of the extended TBI in tropical forests is essential for a pantropical validation of TBI estimates and for identifying the presence of global patterns in decomposition dynamics.

With only Panama serving as a tropical reference (Keuskamp et al., 2013), TBI estimates obtained in this study appeared unusual at times; our estimates of S, particularly those yielded from the physical termite-exclusion treatment (Table 2), ranked the lowest amongst the global dataset, and were less than half the Panama values. Our estimates of k were also unexpectedly low amongst k estimates in the global TBI dataset by Keuskamp et al., (2013), and deviated from the expected relationship between latitude and decomposition rate of a standardised litter (Wall et al., 2008). Temperate sites in the global TBI dataset had higher estimates of k, contrary to what the cooler climate, lower precipitation, and lower diversity of microbial decomposers suggest. For example, the mean k estimate in this study was just half those obtained from temperate forests in Austria and the Netherlands, where k estimates were at least 0.02 day^{-1} (Keuskamp et al., 2013).

Additionally, cross-validation with time-series data revealed that the TBI potentially overestimated a_r by about 25 % (Fig. 4), implying that in our study sites, Rooibos tea was stabilised to a greater extent than green tea, contrary to the original TBI's assumption (Keuskamp et al., 2013). However, we caution that the observed deviation from the TBI's assumption can only be confirmed by obtaining time-series data from additional tropical forest sites. Notwithstanding the possibility of

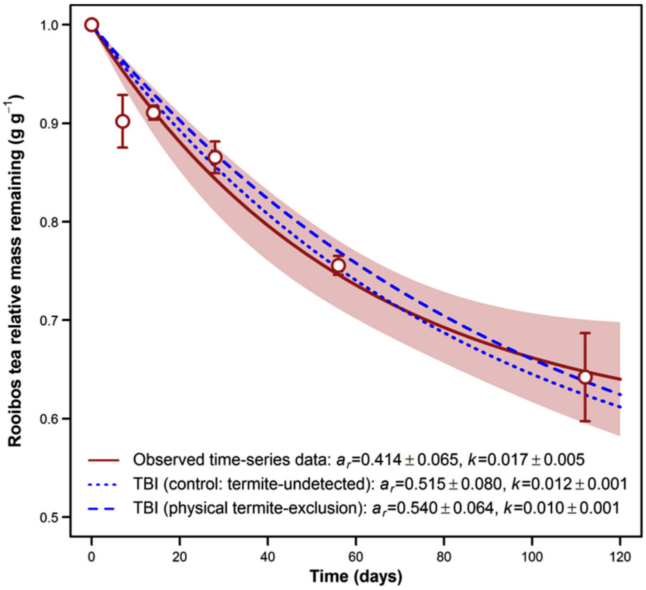

Fig. 4. Comparisons of Rooibos tea mass loss curves predicted by TBI (unpaired approach) with actual time-series data from the BT site, where termite-undetected tea bags were recovered after 7, 14, 28, 56, and 112 days of field-incubation. The mass loss curve for actual observations was fitted to an exponential decay function (Eq. 2), and the shaded regions represent the 95 % confidence intervals. Vertical bars represent standard errors of the means. Data from the CC site is not shown due to the unreliability in model fitting caused by the absence of termite-undetected tea bags for incubation periods exceeding 14 days.

a tea-specific stabilisation rate, estimates of *k* derived from both TBI and time-series data in this study (Fig. 4) were consistently lower, by at least 50 %, than estimates of *k* obtained from Panama, which averaged 0.04 day^{-1} (Keuskamp et al., 2013). This apparent reduction in microbial decomposition rates in our lowland tropical forest sites warrants further regional and pan-tropical validation, to distinguish between a site-specific anomaly and an underlying larger-scale phenomenon. Nonetheless, looking beyond the discrepancy in decomposition *rates*, both the TBI and time-series data here (Fig. 4) are consistent with the consensus that tropical forests drive the largest *extent* of litter decomposition amongst other ecosystems over similar time-scales (Parton et al., 2007). Given that decomposition extent can often provide better insights into decomposition dynamics than early-stage decomposition rates (Prescott, 2005), such as those inferred by *k*, great attention should also be accorded to the estimates of *S*, which regulate organic stabilisation and hence decomposition extent. More importantly, the cross-validation provided the first proof-of-concept that TBI estimates could closely replicate temporal mass loss patterns in tropical forests (Fig. 4), thus serving as an impetus for the widespread deployment of the extended TBI across the tropics.

5. Conclusion

Termites in tropical forests were found to significantly reduce the retrieval rate of TBI parameters. A physical termite-exclusion treatment effectively excluded termites and thus preserved the retrieval rate of TBI parameters. To improve the applicability of the TBI in termite-rich tropical forests, we proposed an extended TBI, with distinct protocols customised for professional scientists and citizen scientists.

For professional scientists, we recommend:

1.) Excluding termites from tea bags with a physical barrier, and subsequently calculate TBI estimates using an unpaired approach.
2.) Deploying supplementary unmodified tea bags, which serve to incorporate termites' contribution in driving tea mass loss. The proportion of termite-detected tea bags will also be reported.

For citizen scientists, we recommend:

1.) Deploying the original TBI and checking retrieved tea bags for evidence of termite-damage. After omitting termite-detected tea bags, TBI estimates will be calculated using an unpaired approach.
2.) Termite-detected tea bags will serve to incorporate termites' contribution in driving tea mass loss. The proportion of termite-detected tea bags will also be reported.

The extended TBI should be deployed at high spatial resolutions to capture the spatial heterogeneity in termite-driven decomposition. By incorporating and inferring both termite- and microbial-driven decomposition, the extended TBI will provide a more comprehensive understanding of decomposition and its drivers in tropical forests.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.pedobi.2020.150639.

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