Teatime in Kruger: Tailoring the application of the Tea Bag Index approach to an African savanna

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Attempts to obtain standardised decomposition data to determine potential drivers of carbon release have evolved from the use of cotton strips and standardised leaf litter mixtures to the most recent Tea Bag Index (TBI). The TBI is an internationally standardised method to collect comparable, globally distributed data on decomposition rate and litter stabilisation, using commercially available tea bags as standardised test kits. As this index was developed as a citizen science project in the northern hemisphere, we aimed to highlight the potential value – and pitfalls – of its application in a subtropical African savanna. We furthermore aimed to expand on existing protocol details and propose amendments to achieve an enhanced understanding of decomposition dynamics across temporal and spatial scales in African ecosystems. Proposed adaptations include extended incubation periods for long-term monitoring studies, the burial of more tea bags to account for potential losses, and the use of additional equipment to enhance effective sampling. These adaptations provide a system-specific protocol which can facilitate studies aimed to understand the interactions between top-down drivers (e.g. herbivory, fire, climate variability) and bottom-up controls (e.g. decomposition) in carbon flux dynamics of savanna ecosystems. Application of the proposed extended protocol in a semi-arid savanna provided results which reinforce the potential value of the TBI in an African context.

Significance:

- The TBI is a relatively easy and cost-effective approach to gather globally distributed data on potential decomposition rate and inherent carbon flux, yet it was developed and primarily tested in boreal and temperate ecosystems.
- The use of more paired tea bag replicates and additional equipment is a viable means to mitigate tea bag losses to several savanna-based agents of disturbance, while enabling confident conclusions made from statistical results and improved estimates of the TBI. High recovery success across disturbance treatments and incubation periods suggest that the TBI can be applied successfully to spatial and temporal decomposition studies.

Introduction

Research on linkages between decomposition and carbon flux is covered extensively for temperate[1,2] and boreal[3,4] ecosystems, whilst studies in tropical and subtropical ecosystems, especially in Africa, remain limited[5,6]. The paucity of information available on these mechanistic relationships in African savannas may be ascribed to complex interactions between top-down (e.g. climate variability, fire, large mammalian herbivores) and bottom-up controls (e.g. soil physical-chemical properties, soil-based microbes and detritivores) that collectively regulate savanna structure and function.[6,7] While governed by various environmental factors, decomposition is primarily regulated by the chemical composition of site-specific leaf litter. Cross-site comparison of natural litter decomposition and intrinsic drivers thereof is subsequently compromised by variability in detrital chemistry.[1,3,4,6,8-10]

Attempts to standardise approaches to examine the role of environmental drivers on decomposition have evolved from the use of cotton strips or natural leaf litter mixtures[1,6,9] to the most recent Tea Bag Index (TBI) approach introduced by Keuskamp et al.[8] This approach is an internationally standardised, user-friendly method to collect comparable, globally distributed decomposition data. The TBI consists of two parameters describing decomposition rate (k) and stabilisation factor (S).[5,8] Standardised test kits include commercially available Lipton® tea bags (i.e. rooibos and green tea).[8] Tea bags were selected based upon differences in chemical composition, with green tea representing high-quality organic matter with low C:N ratios, and rooibos tea a low-quality organic matter with high C:N ratios.[3,8,9] Tea bags therefore act as proxies for labile and recalcitrant compounds in naturally occurring organic matter.[1,8,9]

Assessments of ecosystem functioning, such as the TBI, are becoming increasingly valuable, especially for application in monitoring programmes in areas exposed to increasing intensities of land-use and/or climate change. However, the physical and biological template upon which African systems function varies from most ecosystems in which the TBI has been applied.[1-4] Here, we reflect on applying the TBI in an African context to facilitate decomposition monitoring. Details on (1) an experimental design aimed at elucidating temporal dynamics, (2) tea bag losses due to large mammalian herbivores, fungal infestation and termites, (3) recommended equipment to enhance effective sampling and (4) the potential value in African systems are provided.

Study area

The study was conducted in a semi-arid subtropical savanna at the Nkuhlu exclosures long-term monitoring site (24°58′S, 31°46′E) located in the Kruger National Park, South Africa. This area is characterised by a hot growing season with sporadic precipitation from October to April and a mild to warm, dry non-growing season.[11] Average annual rainfall is 561 mm, with temperatures varying from an average minimum of 5.6 °C in winter to an average maximum of 32.6 °C in summer.[11] Situated on the foot slopes of undulating granitic landscapes, the study site is characterised by sodium-rich, deep duplex soil, referred to as the sodic zone. The plant community of the sodic zone is described as a *Sporobolus nitens-Euclea divinorum* Dry Sodic Savanna and is associated with nutrient-rich vegetation.[11] Sodic patches therefore produce palatable, high-quality forage capable of supporting large herbivores, including grazers and mixed-feeders.[11]

Materials and methods

Experimental design

The Nkuhlu exclosures form part of a large-scale, long-term exclusion experiment of ecosystem drivers (i.e. herbivory and fire) to determine their effect on spatial and temporal heterogeneity patterns of vegetation in a semi-arid African savanna.[11] These exclosures consist of three herbivore treatments including (1) a partially fenced area (elephant exclosure), (2) an unfenced area (control) and (3) a fully fenced area (large mammalian herbivore exclosure).[11] As the TBI was developed and applied in systems in which decomposition is dominated by microbial agents[6,12], it does not account for the presence and possible damage caused by savanna-based disturbance agents, such as large mammalian herbivores and termites[6,10,12]. We applied a paired tea bag design which entailed the burial of 20 green and 20 rooibos tea bags in each of the 25 fixed plots across three herbivore treatments (Figure 1) which added up to 1000 experimental tea bags in total.

Decomposition is a time-bound process consisting of different phases[3,9], primarily regulated by the chemical composition of detrital substrate with respect to labile and recalcitrant ratios[5,8]. Due to the dynamic nature of savannas, regulating factors change over time, influencing the rate and extent of decomposition. Temporal comparisons are therefore essential to observe not only progression in decomposition phases, but the influence of site-specific factors thereon.[6,7,9,12,13] Our suggested extended application includes replicates which represent different incubation periods (i.e. 3-, 6-, 9- and 12-months) to capture dynamics in *k* and *S* but also to determine the resilience of the TBI in an unexplored system over the course of a year (Figure 1). All tea bags were buried in January 2019 (summer) and retrieved in intervals of three months, each representing a different season (i.e. April 2019, July 2019, October 2019 and January 2020).

Statistical analyses

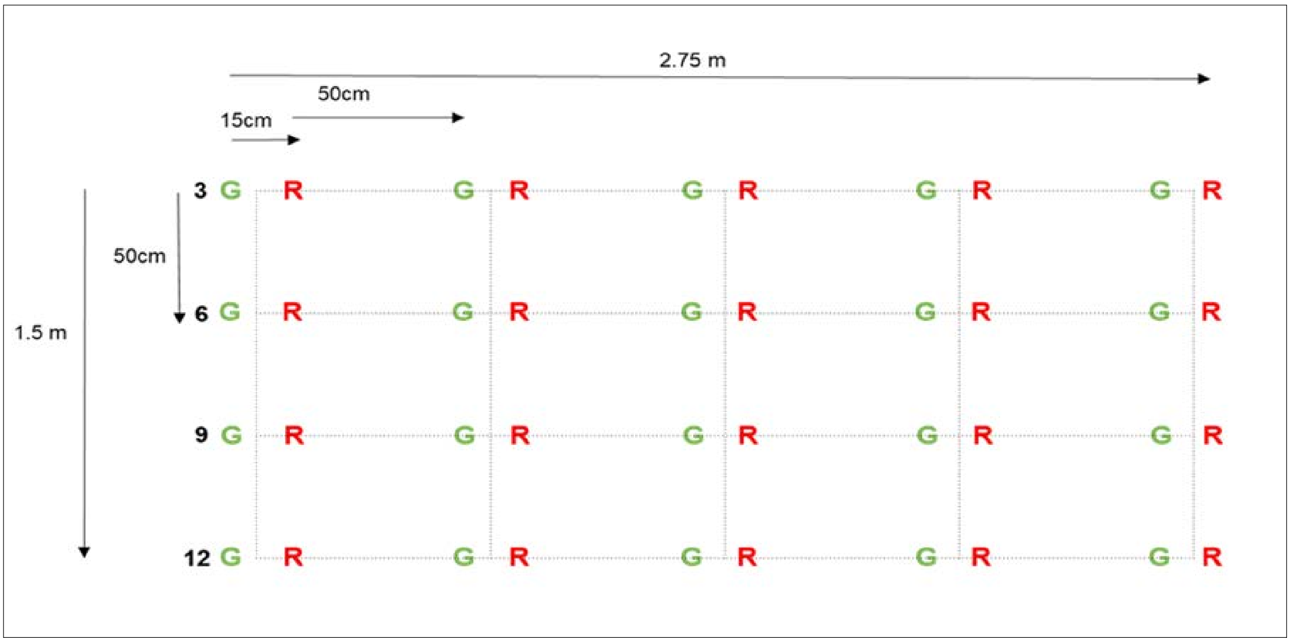
Differences in recovery success of green and rooibos tea bags within each incubation period were tested for significance using independent Student's *t* tests in Microsoft Excel (2013). To test for significant variation in the number of tea bags infested by fungi and damaged by termites across treatments and over the different incubation periods, a one-way analysis of variance (ANOVA) was applied in Paleontological Statistics Software (PAST) and Microsoft Excel (2013).

Results and discussion

The overall recovery success, irrespective of herbivore treatment and incubation, was 78.5%. Results did not reveal significant differences in recovery between green and rooibos tea among most treatments (Figure 2), although significantly fewer rooibos tea bags could be retrieved after the 6-month incubation period (Figure 2).

A finely grained black fungus was present on 42% of recovered green tea bags, while no rooibos tea bags were infested. Fungal infestation showed no significant difference between incubation periods ($p=0.897$). Fungal infestation and damage by termites were significantly lower in the control site ($p<0.001$, Figure 3a and $p=0.003$, Figure 3b). Rooibos tea bags were significantly more damaged by termites than were green tea bags ($p=0.033$).

From our results, it is evident that the presence of large mammalian herbivores complicates the retrieval of tea bags. This could be ascribed to difficulty in finding markers, because the use of above-ground markers is not recommended in areas with high mammal activity. Moreover, detritivore activity in the form of termites has a significant impact on the longevity of tea bags. To account for termite-based damage, Teo et al.[6] suggested the use of physical and/or chemical barriers (e.g. metal mesh and termiticide), although such applications may impede the practicality and standardisation of the TBI and furthermore reduce its effectiveness in measuring decomposition by altering regulatory constituents[12]. The seemingly high number of replicates used in our small-scale investigation successfully buffered the tea bag losses ascribed to animal, insect and fungal activity. Sufficient recovery (78%) with little differences between green and rooibos tea bags will lead to confident conclusions made from estimates of the TBI.



G, green tea bag; R, rooibos tea bag

Figure 1: Experimental plot layout consisting of a paired tea bag design across four incubation periods.

Table 1: Application of the original Tea Bag Index (Keuskamp et al.[8]) and extended version with potential pitfalls and recommendations for African studies

Preparations		
Keuskamp et al.[8]	**Potential pitfalls for African study**	**Recommendations**
Order tea bags (Lipton® green and rooibos) – Note that woven nylon mesh tea bag production halted in 2017 and was replaced with non-woven polypropylene tea bags. Measure initial weight of tea bag and subtract weight of empty tea bag. Mark tea bag labels using permanent marker.	Lipton tea bags need to be shipped from the Netherlands, so logistic delays should be expected. Marked labels may deteriorate when buried. Dry organic material can absorb moisture, altering the initial recorded weight.	Order non-woven polypropylene tea bags (Green -EAN 87 22700 05552 5 / EAN 8714100770542 & Rooibos -EAN 87 22700 18843 8) at least 3 months prior to the onset of the experiment. Cover marked labels with transparent adhesive tape to preserve the markings. Store weighed tea bags at room temperature in airtight containers. Mark cupcake holders and brown paper bags (i.e. used for weighing and storage of retrieved bags) in accordance with individual tea bags.
Equipment		
Keuskamp et al.[8]	**Potential pitfalls for African study**	**Recommendations**
Permanent marker Small spade Above-ground markers (e.g. stick or plastic marker)	Site-specific conditions (i.e. animal activity, compacted soil and high herbaceous biomass) require specialised equipment to minimise tea bag losses.	Transparent adhesive tape Airtight plastic containers GPS Metal detector Rope quadrant (conforming to experimental plot layout) Flat-head shovel Custom-made auger with 8-cm marker (height = 11 cm; diameter = 4.5 cm) Hammer Metal washers (galvanised steel; 1.5 cm diameter) – 1 per tea bag Resealable plastic bags (3.5 x 2.5 cm) – 1 per metal washer Brown paper bags (18 x 8.5 cm) – 1 per tea bag Cupcake holders (standard size – 5.5 cm bottom diameter) –1 per tea bag Sieve (45 cm diameter; 3.5 mm mesh) Portable carrier (field equipment and tea bags) Pharmaceutical tablet holders (10 mL)
Experimental design		
Keuskamp et al.[8]	**Potential pitfalls for African study**	**Recommendations**
Green and rooibos tea bags buried pairwise (15 cm apart) at 8 cm depth. Between 5 and 32 pairs per site (1 m apart). Recommended incubation period of 90 days.	Potential animal and termite activity might lead to tea bag losses, reducing the effectivity of few replicates. Decomposition processes and savanna systems are dynamic, necessitating extended study periods.	1000 non-woven polypropylene tea bags (500 green and 500 rooibos) buried pairwise (15 cm apart) in five plots (1.5 x 2.75 m) per site. Five sites/treatments were sampled in this study. Plots followed a grid design (four rows, 50 cm apart), representing different incubation periods in 3-month intervals. Rows contained five pairs of tea bags (five columns) buried 50 cm apart at 8 cm depth.

Burial		
Keuskamp et al.[8]	**Potential pitfalls for African study**	**Recommendations**
Bury tea bags pairwise (15 cm apart) at a depth of 8 cm. Ensure labels are visible above the soil and mark the area.	Obstructions (e.g. vegetation, termite mounds or gullies) might impede exact replicates. Soil conditions (e.g. compaction) could make burial of tea bags difficult. The study area is subjected to animal activity, therefore the potential effects this might have on the burial and recovery of tea bags should be considered. Locating plots for retrieval might be difficult.	Use a rope quadrant to produce exact replicates of plots. A custom-made mini auger can be used to create holes to bury and retrieve tea bags in hard soil, also limiting disturbance to surrounding soil. Metal washers in resealable plastic bags and label should be placed directly above tea bags 2 cm below the surface to facilitate location with a metal detector. Completely fill holes after burial to ensure tea bags are not exposed and thus susceptible to removal by animals or abiotic factors such as water run-off or wind. Take GPS coordinates at each plot.

[Keuskamp et al.[8] provide very little detail on the removal, analysis and storage of a high number of replicates.]

Retrieval		
Extended procedure for African study	**Potential pitfalls**	**Recommendations**
Locate plots with GPS coordinates and tea bags with a metal detector. Rope quadrant can be used to determine exact position of tea bags. Remove topsoil with a flat-head shovel to expose the label and/or metal washer. Place mini auger directly above the label and screw into the soil up until the 8-cm mark by striking it with a hammer. Remove tea bag from mini auger and place in corresponding cupcake holder and brown paper bag.	GPS accuracy (5 m radius) impedes exact point location. Disturbance to remaining rows (i.e. incubation periods). High numbers of retrieved tea bags may result in confusing numbers and samples. Ambient temperature and humidity may influence results.	Take a picture of the plot with the rope quadrant present. This might help identify significant structures and ease the location of metal washers. Work from the top of the plot (i.e. first row) down so as to not step on the other incubation rows. Brown paper bags absorb moisture and ensure that the tea bag remains dry and does not attract further fungal and bacterial colonisation.

Sample analysis		
Extended procedure for African study	**Potential pitfalls**	**Recommendations**
Place individual tea bags on a sieve to remove excess soil particles and roots. Place cupcake holders and corresponding tea bags in an oven and dry at 70 °C for 48 h. Weigh empty cupcake holder (to three decimal places) and remember to tare the scale. Cut open the tea bag and pour organic material into the cupcake holder and weigh (to three decimal places). Record weight on the TBI 2.0 NW data sheet **for non-woven** tea bags.	The durability of tea bags decreases with incubation and they can easily tear. Foreign material inside tea bag. Ensure that data are recorded on the correct data sheet as there are two different formats (i.e. one for woven nylon tea bags and one for non-woven tea bags).	Gently remove excess particles and debris from tea bags. Due to insect activity, mostly termites, some bags might have holes (resulting in the loss of material) and others might have foreign material inside which will affect the final weight. These tea bags should not be included in the TBI data sheet as it will affect results. As far as possible, one person should weigh all tea bags to ensure consistency not only in weighing but also in identifying these 'altered' tea bags. Visit www.teatime4science.org to download the correct data sheet.

Storage
After weighing the organic matter, the sides of the cupcake holder containing the organic matter should be folded and placed in a correspondingly labelled pharmaceutical tablet holder and stored at room temperature for potential use in the future.

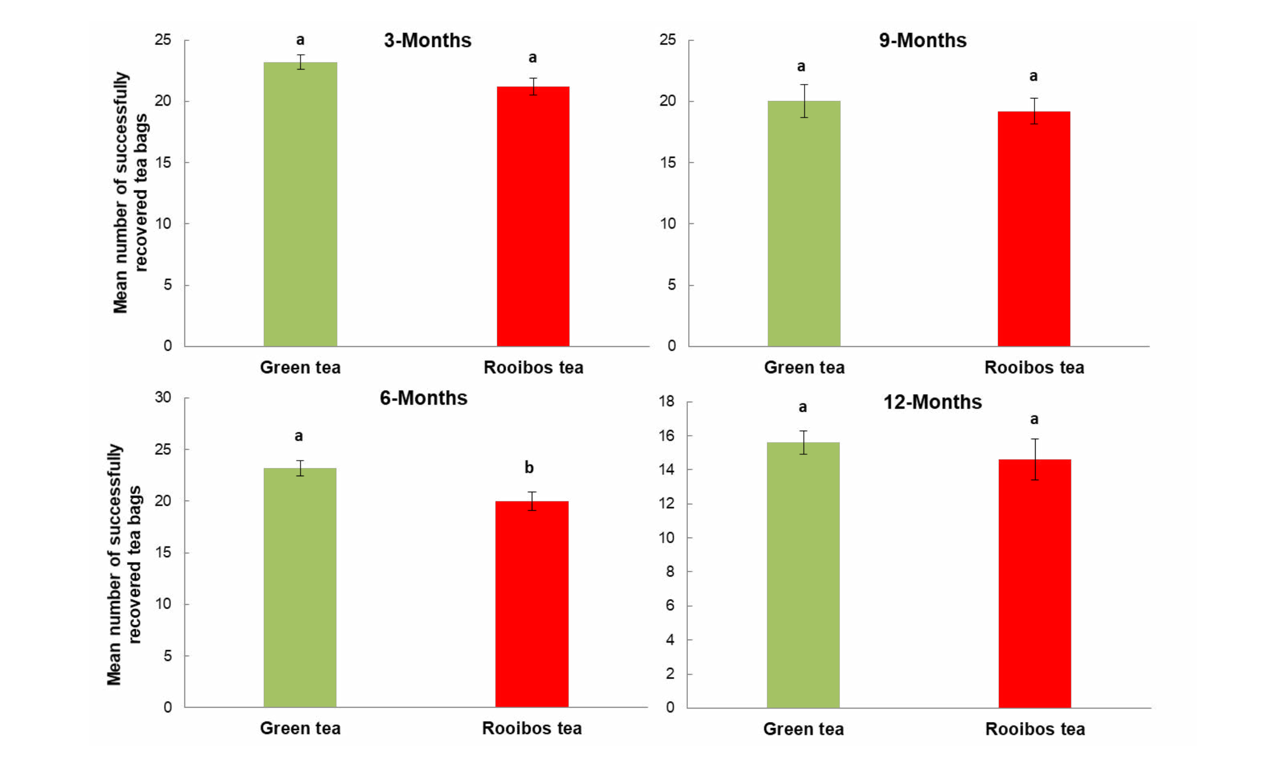
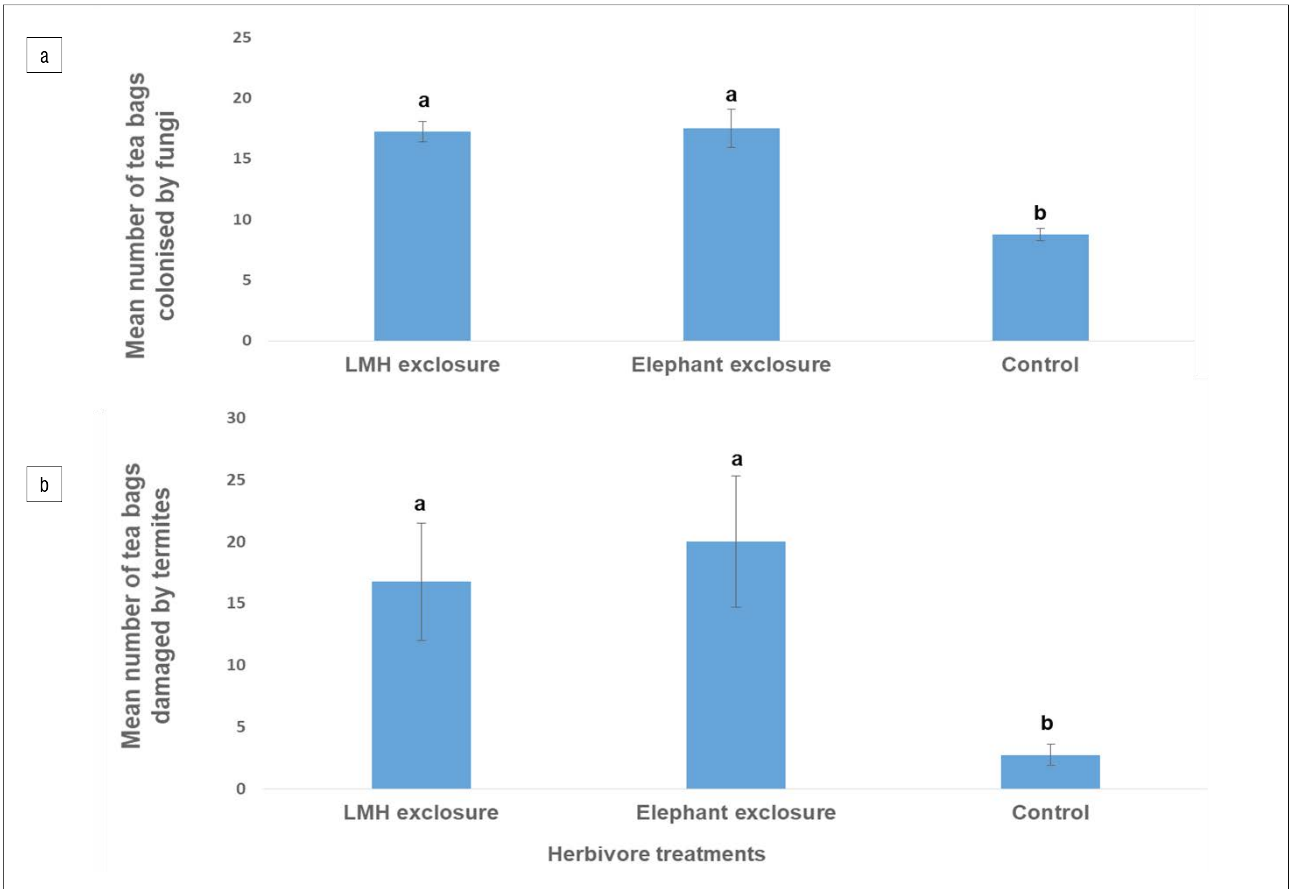


Figure 2: Mean number of successfully recovered tea bags over four incubation periods. Significant differences ($p<0.05$) between tea bags are indicated with different lowercase letters.



LMH exclosure, all large mammalian herbivores excluded; Elephant exclosure, elephants (and giraffes) excluded; Control, all herbivores present

Figure 3: Mean number of tea bags (a) infected by fungi and (b) damaged by termites across herbivore treatments. Significant differences ($p<0.05$) between herbivore treatments are indicated by different lowercase letters.

Value of the TBI in an African context

Decomposition is central to the effective functioning of terrestrial ecosystems as it forms the link between above- and belowground nutrient cycling.[3,4,6,9,14,15] Yet, little is known about site-specific disturbance effects (i.e. herbivory and fire) on decomposition and carbon flux. TBI-based decomposition studies can provide valuable information on the extent of these disturbances and their potential effect on essential ecosystem processes.[1,5,9,14,15] The TBI approach has been developed to remove the subjectivity involved in using site- and species-specific litter, only providing information pertaining to the potential decomposition capacity (i.e. deduced from standardised litter) of a specific site or system based on process-driven soil functions.[1,8,9] However, despite the major differences in chemical composition of standardised (e.g. tea) and local litter, both respond in similar ways to environmental drivers.[1,9] The TBI is therefore able to identify and examine environmental drivers of decomposition without ambiguous effects of site-specific litter, serving as a reference which facilitates data comparison across spatial scales.[1,3,5,8,9]

Despite growing concerns about climate change and increased atmospheric CO_2 levels, research on decomposition and soil carbon flux remains limited for Africa.[5,10] Necessary resources required for such studies are often unavailable in many African countries. However, citizen science projects, such as the TBI, have become a useful tool to facilitate ecological research due to relatively easy application and public engagement (e.g. Teatime 4 Science, http://www.teatime4science.org/).[1,5,8,9]

With the aim to promote efficient decomposition studies, we therefore suggest the application of this extended TBI approach for at least a 9-month duration together with application-based amendments as set out in Table 1. The TBI remains a relatively easy and cost-effective approach which greatly reduces the amount of data to be collected in normal litterbag decomposition studies. As such, this method is more conducive to both the available resources and prevailing environmental conditions in Africa.

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Competing interests

We declare that there are no competing interests.

Authors' contributions

L.L.E.: Methodology, data collection, sample analysis, data analysis, validation, data curation, writing – the initial draft, writing – revisions, project leadership. H.v.C.: Methodology, data collection, sample analysis, data analysis, validation, data curation, writing – the initial draft, writing – revisions, project leadership, student supervision, project management. F.S.: Conceptualisation, writing – the initial draft, writing – revisions, project leadership.

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