Ecology Letters

WILEY

ECOLOGY LETTERS

TECHNICAL NOTE OPEN ACCESS

The Assumptions of the Tea Bag Index and Their Implications: A Reply to Mori 2025

Judith M. Sarneel[1] | Jeff W. Atkins[2] | Laurent Augusto[3] | Janna M. Barel[4] | Sarah Duddigan[5] | Nicolas Fanin[3] | Mariet M. Hefting[6] | Jonas J. Lembrechts[4] | César Marín[7] | Marshall D. McDaniel[8] | Leonardo Montagnani[9] | Tina Parkhurst[10] | Matteo Petit Bon[11] | Adriano Sofo[12] | Joost A. Keuskamp[4,6,13]

[1]Department of Ecology and Environmental Science, Umeå University, Umeå, Sweden | [2]USDA Forest Service, Southern Research Station, New Ellenton, South Carolina, USA | [3]INRAE, Bordeaux Sciences Agro, UMR 1391 ISPA, Villenave d'Ornon Cedex, France | [4]Ecology & Biodiversity Group, Department of Biology, Institute of Environmental Biology, Utrecht University, Utrecht, the Netherlands | [5]Department of Geography and Environmental Science, University of Reading, Reading, UK | [6]Systems Ecology, A Life, Vrije Universiteit Amsterdam, Amsterdam, the Netherlands | [7]Centro de Investigación e Innovación Para el Cambio Climático (CiiCC), Universidad Santo Tomás, Valdivia, Chile | [8]Department of Agronomy, Iowa State University, Ames, Iowa, USA | [9]Faculty of Agricultural, Environmental and Food Sciences, Free University of Bolzano, Bolzano, Italy | [10]School of Environmental and Conservation Sciences, Murdoch University, Murdoch, Australia | [11]Department of Wildland Resources | Quinney College of Natural Resources and Ecology Center, Utah State University, Logan, Utah, USA | [12]Department of Agricultural, Forestry, Food and Environmental Sciences (DAFE), University of Basilicata, Potenza, Italy | [13]Biont Research, Utrecht, the Netherlands

Correspondence: Judith M. Sarneel (judith.sarneel@umu.se)

Received: 17 December 2024 | **Revised:** 10 March 2025 | **Accepted:** 11 March 2025

Editor: Lingli Liu

Funding: This work was supported by Svenska Forskningsrådet Formas (2021-02449).

ABSTRACT

Responding to Mori (2025), we discuss that the simplifications and implications of the Tea Bag Index are essential to its ease of use. However, they necessitate careful attention, especially regarding the appropriate incubation time. Aligning with Mori (2025), we call for a deeper understanding of the interpretation of *k_TBI*.

1 | Introduction

The breakdown of organic material is the result of several processes (e.g., leaching, fragmentation, bleaching, enzymatic hydrolysis) that comprise the process of *decomposition*. The relative importance of these processes can change over time and space, across litter types and material fractions within a given litter type, but they all result in loss of organic material from the original unit (e.g., a leaf). A relatively straightforward and common way to study decomposition of plant material is by determining mass loss curves (Wieder and Lang 1982). However, such mass loss curves are laborious and time-consuming to obtain and difficult to compare across ecosystems because unstandardised leaf litter is used.

The Tea Bag Index (TBI) was introduced to provide an easy-to-quantify and standardised proxy for the decomposition process of plant material during early phases of decomposition (Keuskamp et al. 2013). The method consists of incubating a slow-decomposing rooibos and a fast-decomposing green tea as equivalents of mesh bags with local leaf litter. The tea bag types are incubated for 90 days at 8 cm soil depth. Subsequently, the *observed* mass losses of rooibos and green tea are evaluated using a decomposition *model* with three fractions (i.e., decomposable (labile) material, stabilised material, and recalcitrant material). The model is *parameterised* by using the mass loss observed in rooibos and green tea and the hydrolysable fractions of both tea types (Figure 1). Hence, the TBI provides two proxies of decomposition dynamics: initial decomposition rate (*k_TBI*; Box S1) and stabilisation factor (*S_TBI*, Box S1). The *k_TBI* is used to characterise initial mass loss rates of the hydrolysable fraction of rooibos, whereas *S_TBI* is used to characterise the built up of recalcitrant rest material from the hydrolysable fraction.

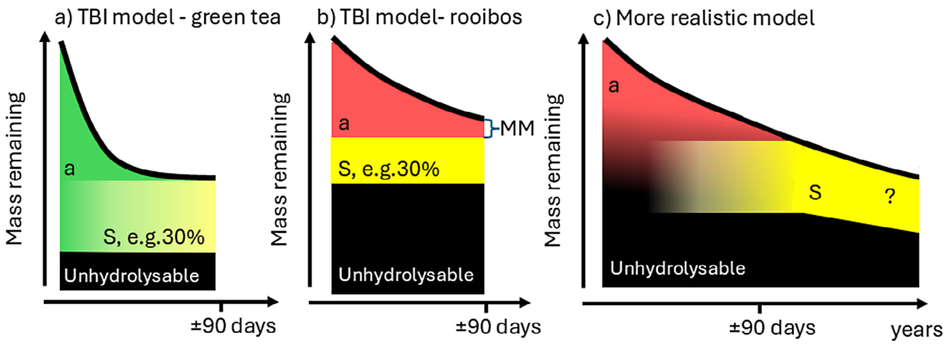

FIGURE 1 | Reasoning of the Tea Bag Index (TBI) model. (a) The TBI is underpinned by a three-fraction decomposition model, with (1) a labile fraction (a; green or red shading) which drives mass loss during early decomposition, (2) a stabilised fraction (yellow) which is derived from incomplete digested compounds from the hydrolysable fraction and (3) an unhydrolysable recalcitrant fraction (black) parameterised by Soxhlet analysis. Using the unique difference in decomposition dynamics between green tea and rooibos, the formation of the stabilised fraction is derived from green tea mass loss after 90 days. It is scaled to the hydrolysable fraction to obtain the stabilisation factor (*S_TBI*). In the TBI, the mass losses from the yellow and black fractions are assumed to be negligible on the short timescales of three months (Assumption 2). (b) To obtain fraction *a* for rooibos, *S* is scaled to the hydrolysable fraction of rooibos and mathematically assumed to form instantaneously (Assumption 3). The *k_TBI* is subsequently estimated from the observed mass loss of rooibos, provided that rooibos has not yet reached its asymptote (Assumption 1), which can be quantified by calculating a mass margin (MM). (c) TBI is a simplification of the reality, where the difference between the hydrolysable and unhydrolysable fraction is not as strict, unhydrolysable material decomposes from the start and can create rest products that are equivalent to hydrolysable material. Hence, TBI does not predict long-term decomposition dynamics (Sarneel et al. 2024).

In a recent analysis of the TBI, Sarneel et al. (2024) showed that, globally, *k_TBI* and *S_TBI* are negatively correlated, although certain environmental conditions (vegetation type and/or climate) can induce deviations from this general trend. In a response to the TBI method in general and to Sarneel et al. (2024) in particular, Mori (2025) pointed out that the correlations between *k_TBI* and *S_TBI* and deviations from the relation reported by Sarneel et al. (2024) should be interpreted cautiously because

Aspect 1: A mathematical dependence of *k_TBI* on *S_TBI* (derived from transferring *S_TBI* from green tea to rooibos) could bias correlation analysis.

Aspect 2: Fundamental differences in decomposition dynamics between both litter types resulting in different responses to environmental conditions could cause deviations from the general negative trend between *k_TBI* and *S_TBI* observed in Sarneel et al. (2024).

Aspect 3: A deviation between *k_TBI* and the observed initial decomposition rate, as derived from mass loss curves of rooibos of ca. 90 days (*k_real*; Box S1).

With this work, we aim to contribute to the discussion on how and when the TBI should or should not be used by first examining its assumptions and subsequently by discussing the aspects raised by Mori (2025). To this end, we expanded the data set of 21 laboratory TBI timeseries incubations of tea used by Mori (2025) with nine unpublished time series (Table S1). With this expanded data set, we followed the procedure outlined by Mori (2025) and calculated *k_TBI* and *Asymptote_TBI* predicted by the TBI at 90 days as well as the observed initial decomposition rate and asymptote (respectively, *k_real* and *Asymptote_real*; Box S1). We related the predicted and observed parameters to each other and investigated what determined the reliability of the prediction (for details on the methodological approach, see Appendix S1). Since Mori's aspects tie to assumptions underlying the TBI, we first discuss these assumptions and conclude by responding to Mori (2025) explicitly.

Assumption 1. The acid unhydrolysable fraction does not decompose within 90 days.

Although parts of the lignified fraction can decompose in 90 days, initial decomposition rates are primarily driven by the loss of the labile, hydrolysable material fraction (Hall et al. 2020; Yi et al. 2023). Yet, the distinction between fractions may be less strict in practice, as partial digestion of the recalcitrant fraction can generate hydrolysable compounds (Aswin et al. 2024). Therefore, decomposition rates derived from timeseries data (TBI or otherwise) integrate mass loss rates of different fractions and processes. Consequently, higher decomposition rates can be observed in shorter time series (Figure 2a), as they primarily capture the rapid loss of labile material, whereas longer time series increasingly reflect the slower degradation of recalcitrant material. This means that timeseries observations of mass loss of rooibos tea may be incompatible with TBI (that aims to model the hydrolysable fraction) when Assumption 1 does not hold. The violation of Assumption 1 can be recognised by negative *S_TBI* values (suggesting unhydrolyzable fraction decomposition), which was observed in 2.37% of the analysed pixels in Sarneel et al. (2024), with a minimum of $S\_TBI = -0.16$ (Figure S3). Although core to the TBI framework, it is often overlooked that longer periods are unsuitable for calculating the TBI decomposition parameters. To prevent too long incubations, Sarneel et al. (2024) restricted incubation duration (45–135 days).

Assumption 2. An incubation of 90 days allows green tea to reach stabilisation (*S_TBI*) and is sufficient for rooibos to reflect initial decomposition rate (*k_TBI*).

The slowly decomposing rooibos and the rapidly decaying green tea differ in their decomposition dynamics (Figure 1).

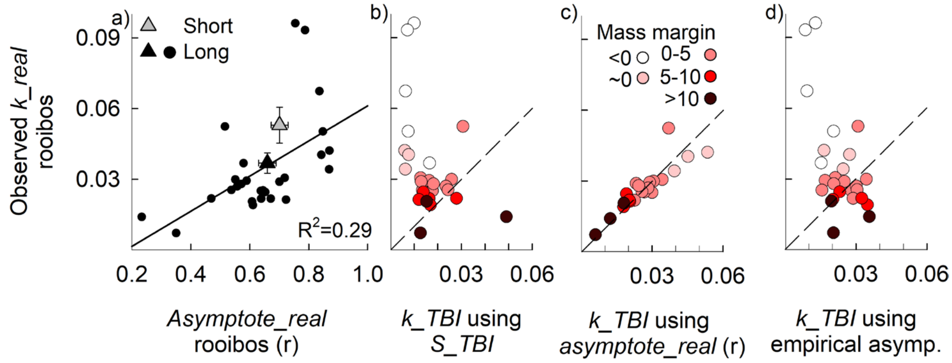

FIGURE 2 | The importance of the asymptote for *k_real*. The observed asymptote (*asymptote_real*) and observed initial decomposition rate (*k_real*) correlate positively in timeseries of rooibos tea (a). Triangles indicate means of the 24 successful fits and show that fits from shorter timeseries (60 days; triangle) result in a higher observed *asymptote_real* and *k_real* compared to the longer timeseries (90–120 days). Error bars are SE. Despite the relationship between the mass loss in green and rooibos (suggesting transferability of stabilisation, Figure S2), *S_TBI* does poor in predicting the observed *k_real* in rooibos (b), especially when mass loss margins (indicated by dot colour) are small. The dashed line indicates the 1:1 line. Small mass margins suggest that rooibos has approached its asymptote at 90 days (Assumption 2; Figure S1), and those observations hence fall outside the TBI framework. Using the observed *asymptote_real* of rooibos to calculate the *k_TBI* highly improves the predictive power (c), suggesting the sensitivity of *k_TBI* to estimation of the asymptote. However, using the empirical relation between the asymptotes of green tea and rooibos (Figure S2; Box S1) to predict the rooibos asymptote does not improve the predictive power of *k_TBI* (d).

After an incubation of ca. 90 days, the mass loss of rooibos represents the initial decay rates, whereas the mass loss of green tea represents an asymptote (Figure 1a,b). As Mori (2025) points out (Aspect 2) the mass loss of green tea and rooibos differ in their decomposition dynamics. Assumption 2 is needed to interpret this difference but, consequently, an incubation that is too long will result in rooibos reaching its asymptote, whereas green tea may not reach its asymptote when the incubation is too short. The latter will result in overestimation of *k_TBI*, and a positive correlation between *k_TBI* and *S_TBI*. Using the extended time series data, we quantified how close the TBI measurement was in relation to reaching the asymptote in both rooibos and green tea. To this end, we calculated the mass margin (Box S1) for both rooibos and green tea. To align Assumption 2, a TBI measurement would require a small mass margin for green tea and a large one for rooibos. We indeed observed a small mass margin for green tea (on average 0.2% ± 2.7% SD of the initial dry tea mass; Figure S1a) but for rooibos, a larger variation in the mass margin was observed. Here, the differences between the predicted and observed initial decomposition rates decreased with increasing mass margins, suggesting a negligible differences at mass margins > 5%–10% (Figures 2b and S1b). Calculating the mass margin for rooibos (Box S1) can quantify if this assumption is met. We suggest maintaining a mass margin of > 10% for rooibos. Though smaller margins may suffice (Figure 2), 10% provides a clear benchmark while remaining measurable in terms of mass loss precision.

In conclusion, the standard TBI incubation duration is suitable for green tea to reach its asymptote, yet it appears to be unsuitable to reliably calculate *k_TBI* on certain occasions (Figures S5 and S6). However, in the data set of Sarneel et al. (2024), we could not find clear environmental conditions that would more frequently result in violation of this assumption (i.e., mass margin rooibos < 10%; Figure S7). We reproduced the results of Sarneel et al. (2024) by including only rooibos measurements that likely met Assumption 2 (with mass margins > 10%; including 66.4% of the tea bag incubations). This showed that, despite some changes in the absolute range of *k_TBI*, the overall patterns remained the same (Figures S5 and S6). However, given that the 90 days may be too long in one third of the considered measurements, a careful consideration of the incubation duration is needed (Box S1).

Assumption 3. The stabilisation factor scales with the hydrolysable fraction and can hence be transferred across litter types.

The literature on 'limit factors' suggests that stabilisation factors may scale with the chemical composition of the leaf material, since limit factors bear conceptual similarity to *S_TBI*. Nevertheless, Mori (2025) suggested that this is not the case (Aspect 3), since the asymptote predicted by the *S_TBI* does not correlate 1:1 with the observed *asymptote_real* in the rooibos time series. However, the significant relationship between the observed *asymptote_real* of green tea and rooibos ($F_{1,25} = 39.0$; $p < 0.001$; $R^2 = 0.59$) suggests that stabilisation factors of rooibos and green tea do scale. The divergence from the 1:1 line could be due to the TBI parameterisation, although using the parameterisation by Hayes et al. (2024; Figure S3) did not improve the predictions. Alternatively, it could be that the stabilisation of green tea may not scale identically with stabilisation in rooibos. When using the observed *asymptote_real* of rooibos instead of *S_TBI*, a 1:1 correlation between the observed *k_real* and *k_TBI* was found (Figure 2c; Box S1). This suggests a high sensitivity of *k_TBI* to asymptote estimations. As an alternative way to predict the asymptote of rooibos (Mori, pers. commun.), we used the empirical relationship between the remaining mass fraction of green tea (the asymptote of green tea in TBI) and the *asymptote_real* of rooibos ($F_{1,25} = 29.8$; $p < 0.001$; $R^2 = 0.54$; Figure S2). This, however, did not increase the predictive power of *k_TBI* (Figure 2d).

While this analysis supports the assumption that *S_TBI* is a parameter that can be transferred across litter types, it also shows that (1) transferring *S_TBI* underpredicts the asymptote of rooibos and that (2) the determination of the initial decomposition rate is sensitive to the asymptote. Nonetheless, rooibos may reach its asymptote on timescales where the recalcitrant material will also start contributing more significantly to mass loss (see Assumption 1). This impairs the estimation of a stabilisation factor for rooibos and hampers comparisons of predicted and observed parameters. Further, *S_TBI* is mathematically implemented at the start of the decomposition, ignoring that at the incubation time when the TBI is calculated, stabilisation may not be completed yet. This would lead to faster *k_TBI* compared to *k_real*. In the time series, however, *k_TBI* underpredicts *k_real* (Figure 2a), making it unlikely that this is a major issue. Last, it should be stressed that transferring *k_TBI* across litter types is outside the scope of the TBI, as that would imply that all litter types would approach the asymptote at the same time.

2 | Response to Mori (2025), Conclusions and Perspectives

The TBI aims to provide an easily applicable method to gain insight into short-term decomposition dynamics of labile litter fractions and highlights that initial decomposition rates and asymptotes are separate characteristics of the decomposition process. The TBI, however, does not intend to elucidate the dynamics of labile versus recalcitrant litter types nor does it try to obtain site-specific insights in decomposition dynamics of local litter. The strength of the TBI lies in its simple application and its standardisation across time and space. This allowed Sarneel et al. (2024) to describe global patterns of *k_TBI* and *S_TBI* across environmental gradients. By analysing the relationships between each parameter and environmental conditions separately, Sarneel et al. showed an overall negative correlation as well as deviations from this relationship. Mori raised that a mathematical dependence (Aspect 1) as well as observations derived from different litters (Aspect 2) could contribute to these patterns. However, the observed relationship between *asymptote_real* and *observed k_real* (Figure 2a) corroborates that those parameters can be independently influenced by environmental conditions. In addition, as outlined by Mori et al. (2022), the mathematical dependence between *k_TBI* and *S_TBI* would induce a positive correlation, whereas Sarneel et al. (2024) observed that environmental conditions induced a negative correlation between *k_TBI* and *S_TBI*. Aligning with Mori's (2025) Aspect 3, we show that *k_TBI* often provides a poor estimation of *k_real*. We suggest that maintaining a mass margin (Assumption 2) could alleviate this issue, but our data set was too small to support this statistically. We further show that the mismatch between *k_TBI* and *k_real* derives from the high sensitivity of *k_TBI* (but likely *k* values in general) to the estimation of the asymptote.

In conclusion, the ecological interpretation of *k_TBI* calls for further investigation as it does not necessarily reflect the mass loss rates observed in rooibos tea. Whether it reflects the mass loss rate of hydrolysable material (as it intends to do) would require advanced chemical analysis. Yet, comparing litter types could benefit from considering which process drove the observed mass loss, especially since the initial decomposition rate and the asymptote are likely influenced by different environmental factors. As showcased in Sarneel et al. (2024) and here, separating these parameters can provide valuable insights into decomposition.

Author Contributions

J.M.S. wrote the original draft with significant input from all other coauthors.

Acknowledgements

We acknowledge the students of the terrestrial biogeochemistry course in 2019 for contributing incubation timeseries data of tea, T. Mori for providing data and the research Council Formas (2021-02449) for funding.

Data Availability Statement

The data added to the data described in Mori (2025) is available on zenodo https://zenodo.org/records/15083202.

Peer Review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/ele.70117.

References

Aswin, T., M. Hedenström, T. Sparrman, M. B. Nilsson, J. Schleucher, and M. Öquist. 2024. "Unraveling the Dynamics of Lignin Chemistry on Decomposition to Understand Its Contribution to Soil Organic Matter Accumulation." *Plant and Soil*. https://doi.org/10.1007/s11104-024-07066-y.

Hall, S. J., W. J. Huang, V. I. Timokhin, and K. E. Hammel. 2020. "Lignin Lags, Leads, or Limits the Decomposition of Litter and Soil Organic Carbon." *Ecology* 101, no. 3: e03113.

Hayes, E. B., C. E. Norris, and J. P. Volpe. 2024. "A Field Assessment to Validate the Assumptions of the Tea Bag Index (TBI) as a Measure of Soil Health." *Applied Soil Ecology* 195, no. 4: 105256. https://doi.org/10.1016/j.apsoil.2023.105256.

Keuskamp, J. A., B. J. J. Dingemans, T. Lehtinen, J. M. Sarneel, and M. M. Hefting. 2013. "Tea Bag Index: A Novel Approach to Collect Uniform Decomposition Data Across Ecosystems." *Methods in Ecology and Evolution* 4, no. 11: 1070–1075. https://doi.org/10.1111/2041-210X.12097.

Mori, T. 2025. "Tea Bag Index Revisited: Risks of Misleading Decomposition Patterns." *Ecology Letters*. https://doi.org/10.1111/ele.70010.

Mori, T., R. Nakamura, and R. Aoyagi. 2022. "Risk of Misinterpreting the Tea Bag Index: Field Observations and a Random Simulation." *Ecological Research* 37, no. 3: 381–389. https://doi.org/10.1111/1440-1703.12304.

Sarneel, J. M., M. M. Hefting, T. Sandén, et al. 2024. "Reading Tea Leaves Worldwide: Decoupled Drivers of Initial Litter Decomposition Mass-Loss Rate and Stabilization." *Ecology Letters* 27: e14415.

Wieder, R. K., and G. E. Lang. 1982. "A Critique of the Analytical Methods Used in Examining Decomposition Data Obtained From Litter Bags." *Ecology* 63, no. 6: 1636–1642. https://doi.org/10.2307/1940104.

Yi, B., C. Q. Lu, W. J. Huang, et al. 2023. "Resolving the Influence of Lignin on Soil Organic Matter Decomposition With Mechanistic Models and Continental-Scale Data." *Global Change Biology* 29, no. 20: 5968–5980. https://doi.org/10.1111/gcb.16875.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Appendix to: The assumptions of the Tea Bag Index and their implications; A reply to Mori 2024

Judith M. Sarneel, Jeff W. Atkins, Laurent Augusto, Janna M. Barel, Sarah Duddigan, Nicolas Fanin, Mariet M. Hefting, Jonas J. Lembrechts, César Marín, Marshall D. McDaniel, Leonardo Montagnani, Tina Parkhurst, Matteo Petit Bon, Adriano Sofo, Joost A. Keuskamp

Index

Appendix 1: Extended method and analysis on the timeseries dataset

The used dataset consists of 32 timeseries of mass losses observed for rooibos and green tea under different conditions (Table S1). The dataset consists mainly of tea from nonwoven bags, but for 18 time series, the tea was placed in hand made woven bags. Given this heterogeneity, we included all bag types in the analysis.

For each tea type at each condition, we fitted a logistics regression and extracted the observed initial decomposition rate (*k_real*; consistent with the terminology in Mori, 2024) as well as the observed asymptote (*asymptote_real*). We calculated the Tea Bag Index (TBI) for the datapoint that was closest to 90 days. We used Nonlinear Least Squares (R version 4.3.1) to fit the mass fraction remaining by $a*e^{-k*t} + (1-a)$, where *a* is the observed asymptote (*asymptote_real*), *k* is the observed initial decomposition rate (*k_real*) during the time period of the observation (*t;* days). The TBI proxies were fitted following Keuskamp *et al.* (2013), on the timestep closest to 90 days (Table S1).
The relation between the observed *asymptote_real* in rooibos and green tea was tested using a linear model with *asymptote_real* in rooibos as the dependent parameter in R version 4.3.1 (R Core Team 2023). Likewise, the relation between the TBI asymptote of green tea (equalling the mass remaining of green tea at 90 days) and *asymptote_real* in rooibos was determined.

To quantify the sensitivity of *k_real* and *asymptote_real* to the duration of the incubation series, we calculated those proxies on the full time series (90-120 days) as well on time series where we removed all measurements that were longer than 60 days. We averaged *k_real* and *asymptote_real* across all timeseries where fits were obtained in both the longer and short time series (Figure 2a).

For each mass remaining at 90 days, for both the timeseries dataset described here as well as the dataset used by Sarneel *et al.* (2024), we used the mass for rooibos tea observed at the time when *k_TBI* was calculated to estimate how close this observation is to the asymptote (mass margin; Box 1). For the time series dataset, we used the observed asymptote as derived from our nonlinear least squares analysis, and for the dataset in Sarneel *et al.* (2024), we used *S_TBI* to estimate the asymptote for rooibos. In addition, we calculated the difference between *asymptote_TBI* and *asymptote_real* of green tea and between *k_TBI* and *k_real*, to visualize how the mass margin related to the predictive power of the *TBI_S* and *TBI_k*, respectively.

Next to *k_TBI* (Keuskamp et al. 2013), we calculated two other initial decomposition rates using the rooibos mass remaining at ca. 90 days and following the TBI formula's. However, we substituted the asymptote based on *S_TBI* by 1) the observed *asymptote_real* in rooibos, and 2) by an estimated asymptote based on the empirical relation between green tea mass remaining (which equals the asymptote of green tea in the TBI model) and *asymptote_real* in rooibos. The empirical relation between green tea mass remaining and *asymptote real* is described as asymptote_rooibos = 1.135 * mass remaining green + 0.233.

We also explored the role of parameterization by calculating *k_TBI* and *S_TBI* using the hydrolysable fractions described in Hayes *et al.* (2024), with *H* = 0.7967 and 0.6352 for green tea and rooibos, respectively

Table S1: Overview of the dataset presented in Fig 2 and 3. The table indicates the source, a general description of the incubation conditions, the type of tea used (with W for woven, NW for non-woven, PB for plant based, and W/NW tea from nonwoven bags placed in woven bags), if the initial mass of the bags was determined by oven drying (at ca 60/70C) or by subtracting a correction for moisture from the initial mass, the number of replicates per timestep (*n*), the number of timesteps included, and for which duration (in days) the *k_TBI* and *S_TBI* were calculated and lastly, an indication for which of the timeseries the model could not be fitted in R or the calculation of the TBI proxies failed. Sources are 1: Duddigan *et al.* (2020), 2: Keuskamp *et al.* (2013), 3: Mori (2022b), 4: Mori (2022a), 5: Middelanis *et al.* (2023) and 6: Sarneel (unpublished).

Source	General conditions	Mesh	initi al	n	Time steps	TBI duration	Failure
1	20 C, fertile soil	W	dried	3	14	91	
2	15 degrees, forest soil	W	none	6	7	68	
2	25 degrees, forest soil	W	none	6	7	68	
3	Unknown	W/NW *	dried	1	7	90	
3	Unknown	W/NW *	dried	1	7	90	
3	Unknown	W/NW *	dried	1	7	90	
3	Unknown	W/NW *	dried	1	7	90	
3	Unknown	W/NW *	dried	1	7	90	
3	Unknown	W/NW *	dried	1	7	90	
3	Unknown	W/NW *	dried	1	7	90	
3	Unknown	W/NW *	dried	1	7	90	
3	Unknown	W/NW *	dried	1	7	90	
3	Unknown	W/NW *	dried	1	7	90	
4	3C, moist	W/NW *	dried	1	6	90	
4	25 C, dry	W/NW *	dried	1	6	90	fit, k_TBI
4	3C, moist	W/NW *	dried	1	6	90	
4	25 C, dry	W/NW *	dried	1	6	90	
4	3C, moist	W/NW *	dried	1	6	90	
4	25 C, dry	W/NW *	dried	1	6	90	fit
4	3C, moist	W/NW *	dried	1	6	90	
4	25 C, dry	W/NW *	dried	1	6	90	fit
5	14 C, moist	W	dried	3	4	90	fit
5	14 C, moist	NW	dried	3	4	90	fit, k_TBI
6	10 C, moist potting soil	NW	none	5	5	99	
6	20 C, moist potting soil	NW	none	5	5	99	
6	10 C, dry potting soil	NW	none	5	5	99	
6	20 C, dry potting soil	NW	none	5	5	99	
6	18C, potting soil	PB	none	2-4	5	90	
6	18C, potting soil	PB	dried	2-4	5	90	
6	30 C, moist soil/sand	NW	none	8	6	84	
6	15 C, moist soil/sand	NW	none	8	4	84	
6	2 C, moist soil/sand	NW	none	8	5	84	

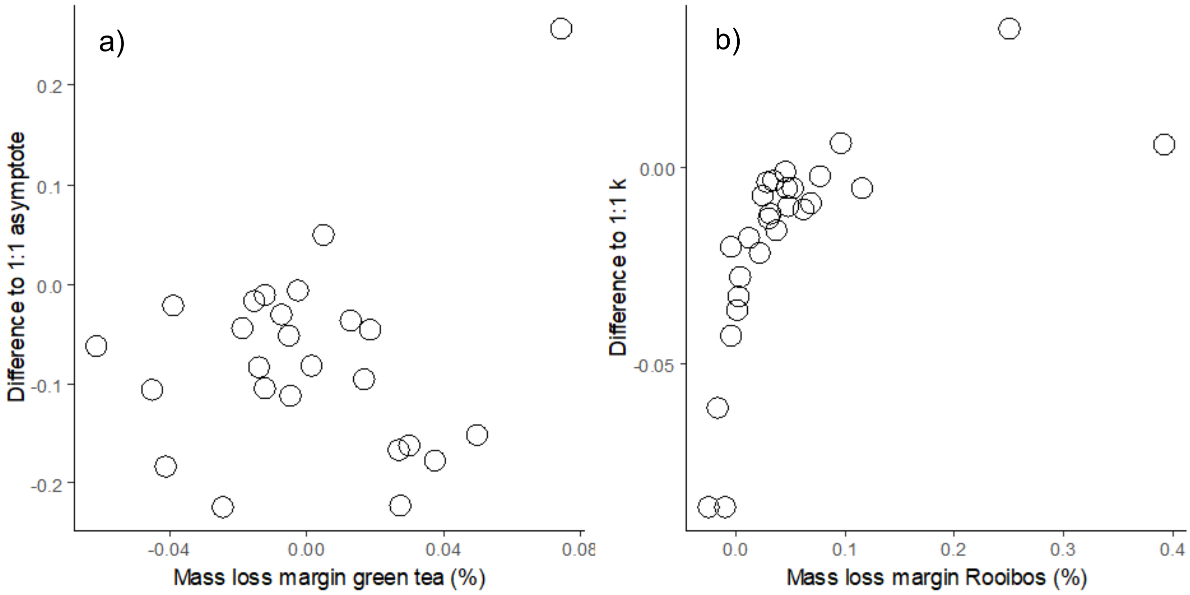

Figure S1: Deviations from the 1:1 line of the TBI-predicted and observed asymptotes of green tea (residues) in relation to the mass margin of green tea (a). In (b) absolute differences between predicted and observed initial decomposition rate of rooibos tea (residues) are given showing in relation to the mass margin of rooibos indicating how far the decomposition curve has approached the asymptote of rooibos tea. In other words, the mass margin indicates whether assumption 2 is violated or not. Because the relation is not linear in b) visual inspection suggest that the mass margin of rooibos requires to be around 10% to predict the observed *k_real*. Note that the scale of a and b are different orders of magnitude.

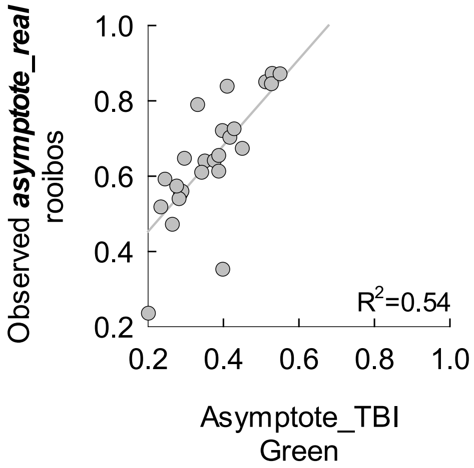

Figure S2: Empirical estimation of the asymptote of rooibos based on the relation between the green tea asymptote as described by TBI (equalling the mass remaining at 90 days) and the observed asymptote in rooibos. The relation is described by asymptote rooibos = 1.135 * asymptote TBI of green tea + 0.233, where the asymptote of green tea is defined by the mass fraction remaining in green tea after 90 days, which, given the small mass margin of green tea is a valid assumption.

Effect of parametrization on the relation between predicted and observed decomposition dynamics.

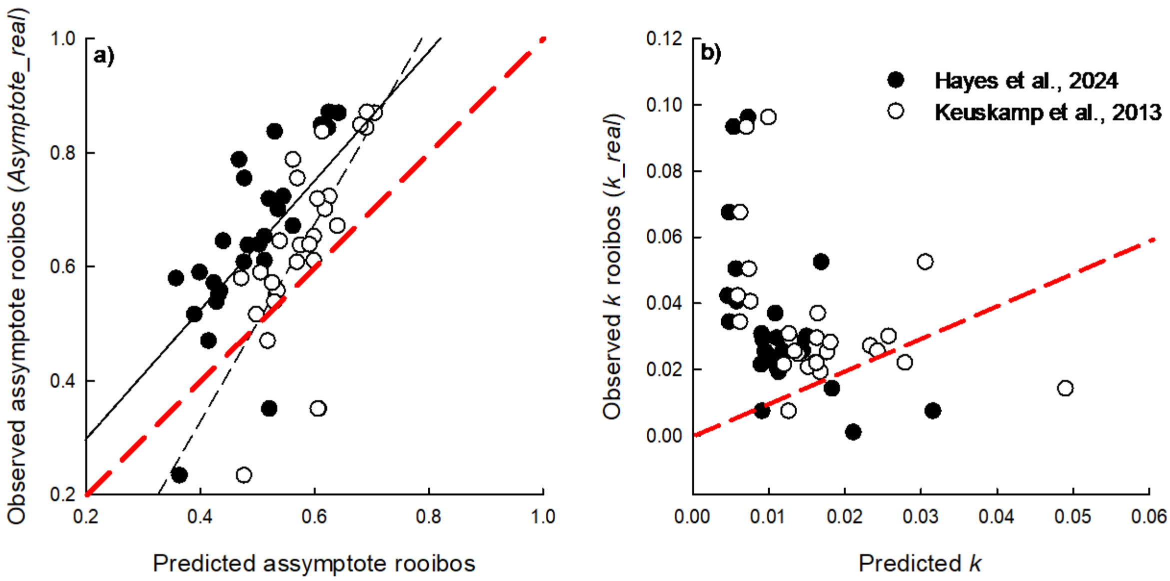

Figure S3: Using a different parameterization of the unhydrolysable fraction of rooibos and green tea does not change the mismatch observed between calculated and observed asymptote (a) and initial decomposition rate (b) of rooibos tea. The predicted asymptote was calculated following the procedure described in Keuskamp *et al.* (2013), either using the original hydrolysable fraction (white dots; H = 0.842 and 0.552 for green tea and rooibos respectively) or using the hydrolysable fraction described in Hayes *et al.* (2024), with H = 0.7967 and 0.6352 for green tea and rooibos respectively. Each point represents one timeseries and the red line indicates the 1:1 line, while the black lines indicate fitted regression lines.

Appendix 2: Re-evaluating the data of Sarneel *et al.* (2024) when keeping a large mass margin for rooibos tea

Since analyses in Appendix 1 suggest that a mass margin of ca 10% is needed for *TBI_k* to reflect the observed *k_real*, we re-ran the analyses presented in Sarneel *et al.* (2024) using only the data where the mass margin for rooibos is >10% (taking into account S_TBI). Removing these observations slightly changed the range of observed values (Figure S3). We explored if there were consistent differences between the two categories of measurements and show both the original figures as well as the updated figures of our re-analysis. In general, we did not find systematic differences that could explain conditions under which the mass margin becomes too small (Figure S4). Re-analysis of the patterns described by Sarneel *et al.* (2024) decreased the absolute values of *k_TBI*, but the patterns across large gradients remained the same (Figure S5, S6).

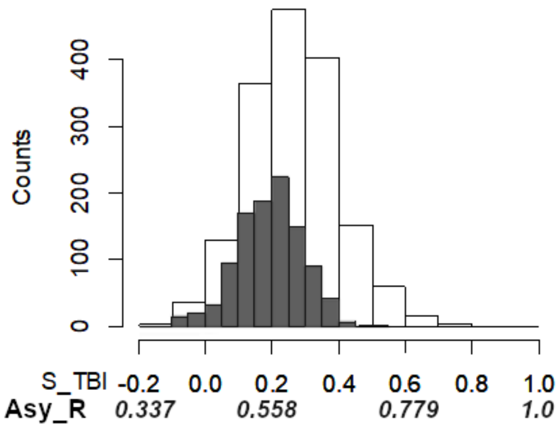

Figure S4: Distribution of S_TBI values in the original dataset of Sarneel *et al.* (2024) indicated with white bars and in the dataset where all the observations with a mass margin of less than 10% in rooibos have been removed (dark shaded bars). On the x-axis, the *S_TBI* values are indicated as well as the associated predicted asymptote of rooibos tea (*Asy_R*) based on this *S_TBI.*

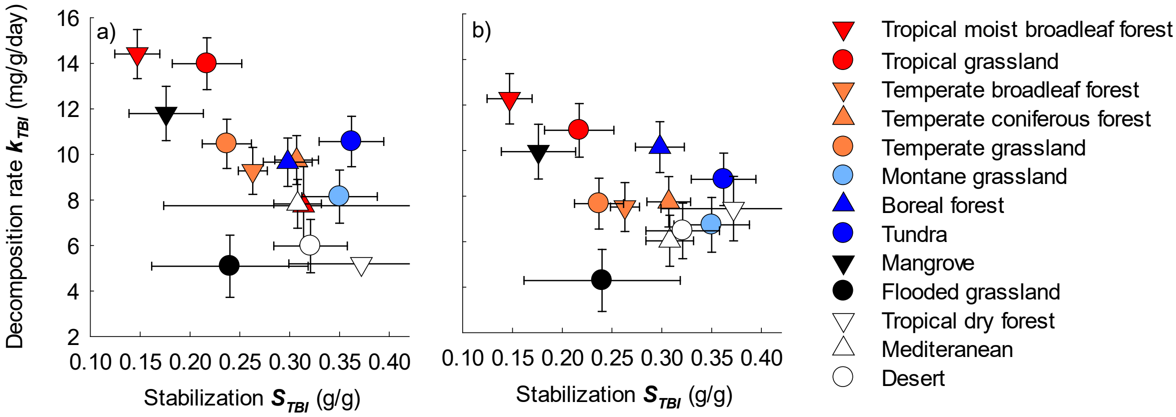

Figure S5: Mean ***k_TBI*** and ***S_TBI*** per biome as presented in a) Sarneel ***et al.*** (2024) and b) when removing measurements with a mass margin smaller than 10% in rooibos. “Colour coding follows main climatic conditions, with red for tropical, orange for temperate, blue for cold, black for wetlands and white for dry ecosystems. Forest biomes are indicated by triangles and low vegetation system by circles. Values shown are corrected for spatial autocorrelation” as described in Sarneel ***et al.*** (2024). Error bars are standard errors. Biome names follow Olson ***et al.*** (2001) with abbreviations as in Sarneel ***et al.*** (2024).

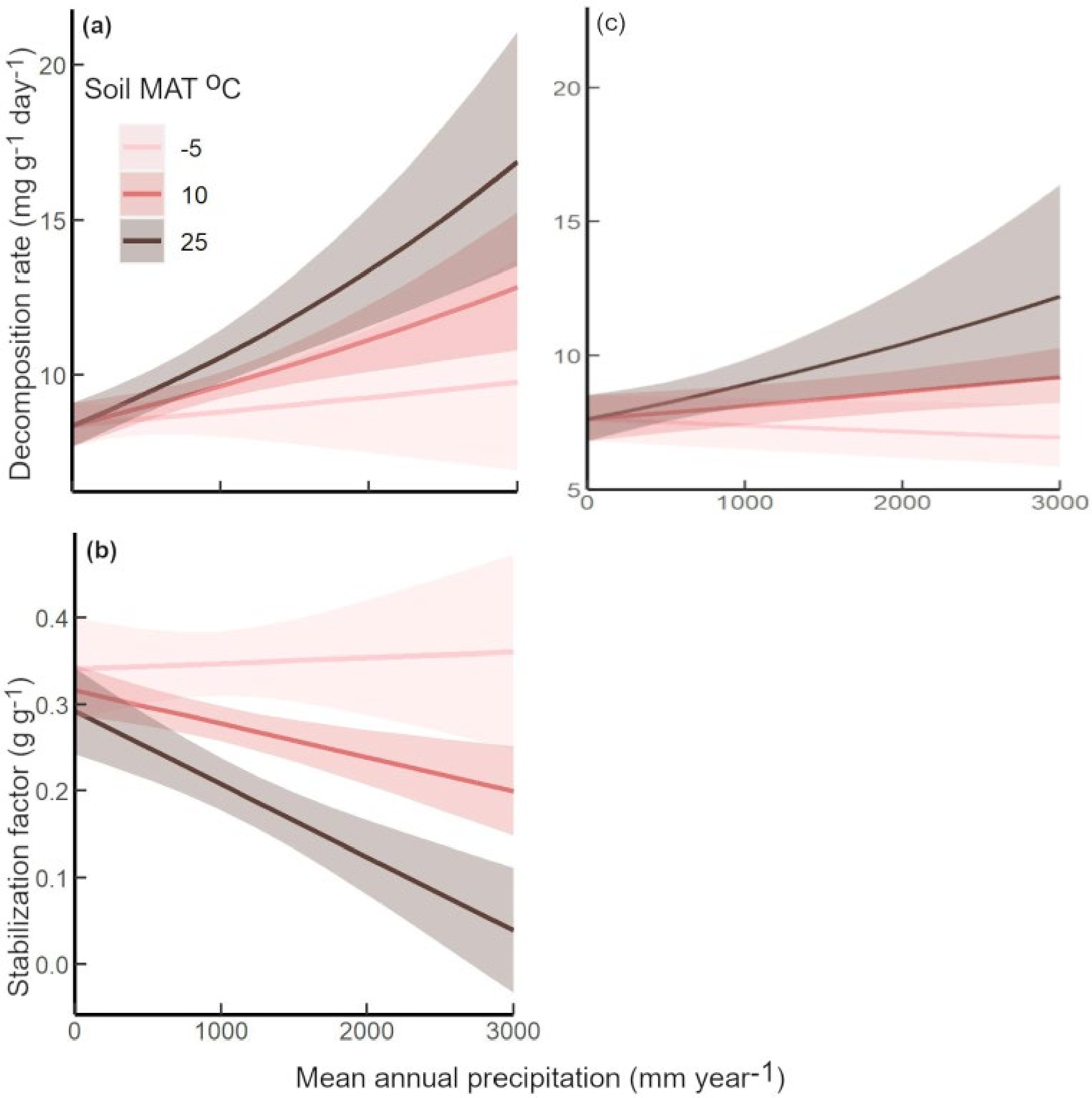

Figure S6: The interaction of both *k_TBI* and *S_TBI* with Mean Annual soil Temperature (MAYT) and Mean Annual Precipitation (MAP) causes decoupling in dryer and colder environments. Relationship between mean annual precipitation (MAP) and *k_TBI* (a) *S_TBI* (b) for different values of mean annual soil temperature, based on the models described in Sarneel *et al.* (2024) and c) for the re-analysis taking only measurements with a large mass margin into account (>10%). Lines indicate the mean and the shaded areas the confidence intervals obtained using ‘predictSE.gls’ in the AICcmodavg package in R.

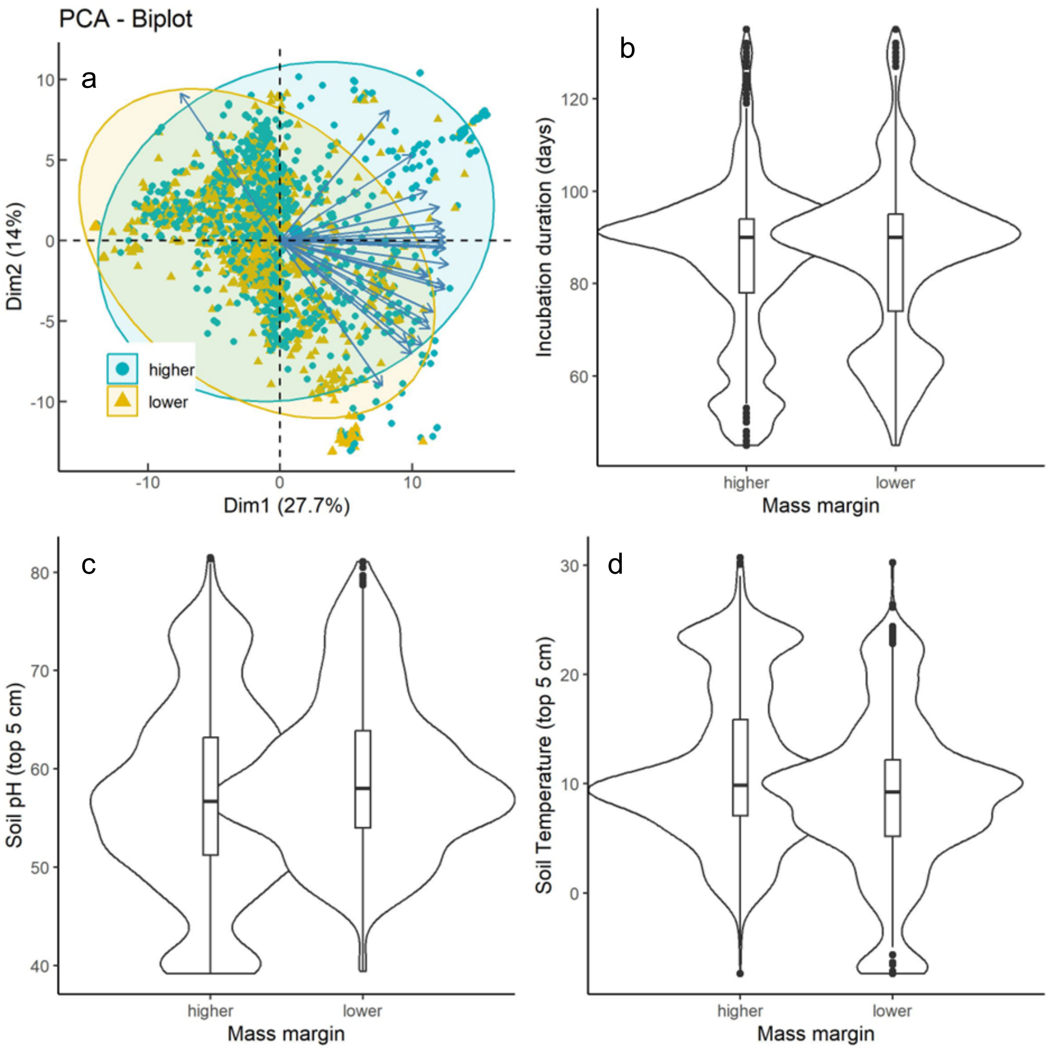

Figure S7: a) PCA plot (See Sarneel *et al.* 2024 for a description of the parameterspace) with yellow points indicating measurements where the mass margin of rooibos was <10% (lower), and blue points for measurements with a mass margin larger than 10% (higher). The distribution and median for b) incubation duration, c) Soil pH in the top 5 cm based on water extractions and derived from https://www.soilgrids.org as well as d) mean annual soil temperature derived from Lembrechts *et al.* (2022). The analysis in Figure S4 and S5 are based on the data in the 'higher' category.

Box S1 **List of terms**

Asymptote_TBI: For rooibos this encompasses the unhydrolyzable fraction plus the stabilized fraction of the hydrolysable fraction (H) rooibos. For green tea, the *asymptote_TBI* equals the mass fraction remaining at 90 days. Conceptually representing (1-a) in eq. 2 and calculated by using the stabilization factor (*S_TBI*):

$$\boldsymbol{Asymptote\_TBI}_{(\boldsymbol{rooibos})} = 0.552 * S\_TBI + 0.448 \quad \text{eq. 1}$$

Asymptote_real: Observed asymptote in timeseries. That is, the measured mass fraction at which the mass loss curve is observed to level off. It can be observed for green tea and rooibos separately. Conceptually representing (1-a) in eq. 2.

Asymptote_empirical: Predicted asymptote of rooibos as derived from the relation between mass fraction remaining of green tea and the observed *asymptote_real* of rooibos tea given by: *asymptote_empirical* = 1.135 * *asymptote_TBI* green tea + 0.233, where *asymptote_TBI* green tea is defined by the mass fraction remaining at the time point of measuring.

Decomposition model: The Tea Bag Index (TBI) is based on the decomposition model for early-stage decomposition of fresh litter described by Wieder and Lang (1982), which characterizes an exponential decay towards an asymptote (eq. 2), reflecting varying decomposition rates among material fractions. As the TBI focuses on the initial phases of decomposition, mass loss is predominantly driven by the rapid decomposition of the most labile fraction, while the rates for more recalcitrant fractions are minimal

$$M_t = ae^{-kt} + (1 - a) \quad \text{eq. 2}$$

Hydrolysable fraction (H): Fraction of fresh litter that are dissolved in the Soxhlet procedure (*H* green tea= 0.842 ; *H* rooibos = 0.552 , Keuskamp *et al.*, 2013). This fraction is interpreted as potentially hydrolysable (labile) and comprises the labile and the stabilized fraction.

***k_TBI*:** Decomposition rate estimated following the TBI method, describing the mass loss dynamics of the labile fraction following eq. 2. In short, it is calculated at 90 days of incubation by scaling S_TBI and resolving eq 2. using the observed mass loss of rooibos. Equivalent to $k1_{TBI}$ (Sarneel *et al*. 2024). For this work, we recalculated *k_TBI*, once by using the *asymptote_real* of rooibos and second by using *Asymptote_empirical*, by substituting those for (1-a) in eq 2.

***k_real*:** Observed decomposition rate derived from time series (Appendix 1) using Nonlinear Least Squares models.

Labile fraction (*a*): Easy to decompose material. Described by decomposition rate constant (*k*). For TBI, *a* is quantified by the mass loss fraction observed in green tea after 90 days.

Mass margin (MM): The difference between the observed mass fraction remaining at 90 days and observed *asymptote_real*, Indication how far decomposition has advanced to the asymptote. Can be calculated for both rooibos and green tea time series as well as field data on rooibos tea when replacing *asymptote_real* by its best approximation (*asymptote_TBI*):

$$\boldsymbol{Mass\ margin} = Mt - Asymptote\_real\ rooibos\ = Mt - Asymptote\_TBI \qquad \text{eq. 3}$$

Stabilized fraction: The part of *H* that becomes stabilized and more recalcitrant to decomposition.

Stabilization factor (*S_TBI*): The stabilized fraction is scaled to the size of the hydrolysable fraction. *S_TBI* is equivalent to S_{TBI} (Sarneel *et al*. 2024) and *S* (Keuskamp *et al*. 2013) and quantified as:

$$S\_TBI = 1 - \frac{a_g}{H_g} \qquad \text{eq. 4}$$

Unhydrolyzable, or acid-insoluble, fraction: The fraction of fresh litter that is not soluble in fat, water or acid and is left after the Soxhlet extraction (green tea: 0.258; rooibos: 0.448; Keuskamp et al., 2013). This material is considered to be recalcitrant to decomposition and contains a high proportion of lignified material.

References

1.

Duddigan, S., Shaw, L.J., Alexander, P.D. & Collins, C.D. (2020). Chemical Underpinning of the Tea Bag Index: An Examination of the Decomposition of Tea Leaves. *Applied and Environmental Soil Science*, 2020.

2.

Hayes, E.B., Norris, C.E. & Volpe, J.P. (2024). A field assessment to validate the assumptions of the Tea Bag Index (TBI) as a measure of soil health. *Applied Soil Ecology*, 195.

3.

Keuskamp, J.A., Dingemans, B.J.J., Lehtinen, T., Sarneel, J.M. & Hefting, M.M. (2013). Tea Bag Index: a novel approach to collect uniform decomposition data across ecosystems. *Methods in Ecology and Evolution*, 4, 1070-1075.

4.

Lembrechts, J.J., van den Hoogen, J., Aalto, J., Ashcroft, M.B., De Frenne, P., Kemppinen, J. *et al.* (2022). Global maps of soil temperature. *Global Change Biol.*, 28, 3110-3144.

5.

Middelanis, T., Pohl, C.M., Looschelders, D. & Hamer, U. (2023). New directions for the Tea Bag Index: Alternative teabags and concepts can advance citizen science. *Ecol. Res.*, 38, 690-699.

6.

Mori, T. (2022a). Is the Tea Bag Index (TBI) Useful for Comparing Decomposition Rates among Soils? *Ecologies*, 3, 521-529.

7.

Mori, T. (2022b). Validation of the Tea Bag Index as a standard approach for assessing organic matter decomposition: A laboratory incubation experiment. *Ecol. Indicators*, 141.

8.

Olson, D.M., Dinerstein, E., Wikramanayake, E.D., Burgess, N.D., Powell, G.V.N., Underwood, E.C. *et al.* (2001). Terrestrial ecoregions of the worlds: A new map of life on Earth. *Bioscience*, 51, 933-938.

9.

R Core Team (2023). R: A language and environment for statistical computing. R Foundation for Statistical Computing Vienna, Austria.

10.

Sarneel, J.M., Hefting, M.M., Sandén, T., van den Hoogen, J., Routh, D., Adhikari, B.S. *et al.* (2024). Reading tea leaves worldwide: Decoupled drivers of initial litter decomposition mass-loss rate and stabilization. *Ecol. Lett.*, 27.