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Is the Tea Bag Index (TBI) Useful for Comparing Decomposition Rates among Soils?

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Abstract: The Bag Index (TBI) is a novel approach using standardized materials (i.e., commercial tea bags) to evaluate organic matter decomposition by determining two indexes: the early stage decomposition constant k (k_TBI) and litter stabilization factor S (S_TBI). k_TBI is defined as the decomposition constant of an asymptote model describing the decomposition curve of rooibos tea, whereas S is the ratio of the stabilized to total hydrolysable fractions of green tea. However, it was recently revealed that both k_TBI and S_TBI deviate from the actual S and k values accurately determined by fitting an asymptote model to the time series mass of green and rooibos teas remaining ($k_fitting$ and $S_fitting$, respectively). Nevertheless, k_TBI and S_TBI, which can be determined in a cost- and labor-effective manner, might indicate the relative values of $k_fitting$ and $S_fitting$ across different soils and be useful for comparative analyses. Therefore, this study investigated the positive correlations of k_TBI and S_TBI with $k_fitting$ and $S_fitting$, respectively, in which case these indexes are useful for comparative analyses. However, the result showed that k_TBI was negatively correlated with $k_fitting$. This study underscores the importance of obtaining time-series data for accurately determining the decomposition constant of an asymptote model describing the decomposition curve of rooibos tea. S_TBI was positively correlated with $S_fitting$, implying that S_TBI can be used as an indicator of S.

Keywords: asymptote model; decomposition constant k; stabilization factor S; Tea Bag Index

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1. Introduction

Decomposition is an essential ecological process with an important role in nutrient recycling and carbon dynamics. Accordingly, various studies have evaluated the impact of anthropogenic environmental changes, including climate change and eutrophication, on litter decomposition rates [1]. Because the impact on decomposition rates is largely influenced by the variability in litter chemistry, which regulates litter decomposition, standardized materials are essential for evaluating the global-scale impact of environmental changes on litter decomposition.

The Tea Bag Index (TBI) was proposed as a novel approach using standardized materials (i.e., commercial green and rooibos tea bags [2]) to evaluate organic matter decomposition. This approach was designed to determine two decomposition indexes, the decomposition constant k (hereafter k_TBI) and stabilization factor S (hereafter S_TBI), which indicate the early decomposition rate and litter stabilization, respectively [2] (see Figure 1). Originally, this approach was proposed for promoting citizen science [2], but researchers are increasingly using this approach for academic studies, including studies to clarify the precise decomposition mechanisms [3–6].

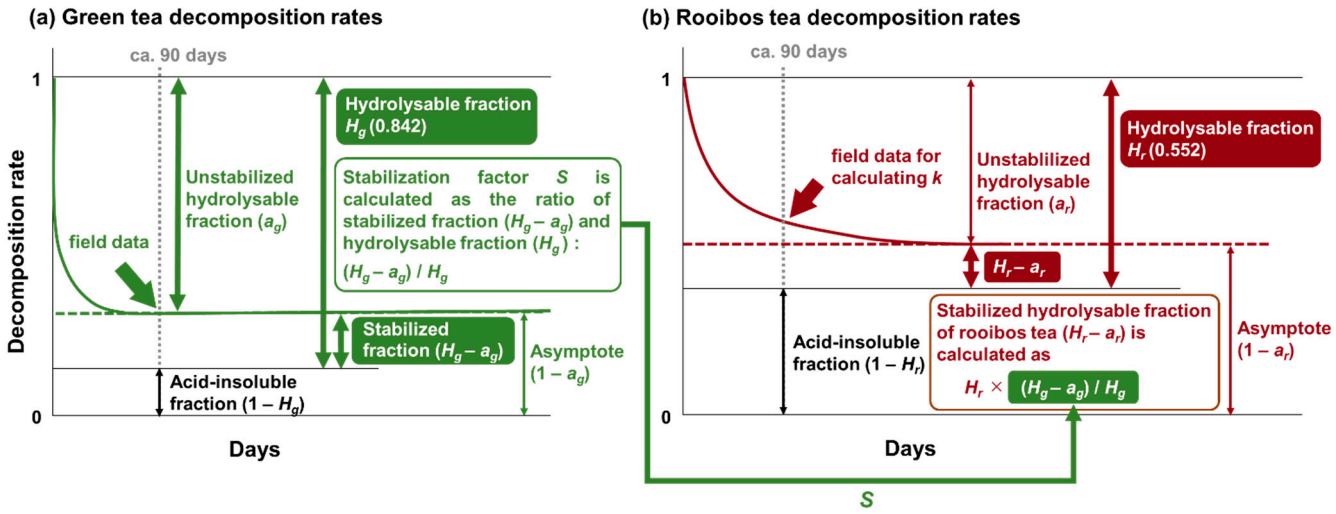


Figure 1. The concept of the Tea Bag Index (TBI) approach. The figure is modified from Mori (2022) [7]. The (**a**) left and (**b**) right figures are the decomposition curves of green and rooibos teas, respectively. a_g and a_r are the unstabilized hydrolysable fractions of green and rooibos teas, respectively. H_g (0.842) and H_r (0.552) are the hydrolysable fractions of green and rooibos teas, respectively. The S in the figure indicates *S_TBI*. The decomposition constant k (*k_TBI*) can be determined by fitting an asymptote model (*Mass of rooibos tea remaining* = $a_r \times e^{-k \times t} + (1 - a_r)$, see Equation (4)), to the mass of rooibos tea remaining at the end of the approximately 90-day incubation.

In the TBI approach, *k_TBI* is defined as the decomposition constant of an asymptote model describing the decomposition curve of rooibos tea (Figure 1b), while *S_TBI* is calculated as the ratio of the stabilized to hydrolysable fractions of green tea. The TBI approach enables determination of *k_TBI* and *S_TBI* using a single pair of mass loss values for the two types of tea during an approximately 90-day incubation (Figure 1). Namely, time-series data are not required. This is possible because the TBI approach assumes that most of the unstabilized hydrolysable fraction of green tea is decomposed within 90 days (the first assumption), and the stabilization factor S (i.e., the ratio of the stabilized to total hydrolysable fractions) of rooibos tea equals that of green tea (second assumption). In the TBI approach, the hydrolysable fractions of green and rooibos teas were divided into unstabilized (i.e., a_g and a_r, respectively) and stabilized (i.e., $1 - a_g$ and $1 - a_r$, respectively) fractions. Using the first assumption, a_g can be approximated to the mass loss ratio of green tea at the end of the approximately 90-day incubation. Therefore, stabilization factor S (*S_TBI*) can be determined using the approximated a_g and H_g (0.842, determined by Keuskamp et al. (2013) [2]) (i.e., $1 - a_g/H_g$, Figure 1). Using the second assumption, stabilized hydrolysable fraction of rooibos tea (i.e., $H_r - a_r$) can be determined by multiplying H_r (0.552, determined by Keuskamp et al. (2013) [2]) by *S_TBI*, and therefore a_r can be calculated as multiplication of H_r by $(1 - S_TBI)$. *k_TBI* can be determined by fitting an asymptote model to the mass of rooibos tea remaining at the end of the approximately 90-day incubation (Figure 1, see also Section 2.2.).

However, Mori (2022) [7] recently reported that these essential premises for determining *k_TBI* and *S_TBI* are not always satisfied by demonstrating that both *k_TBI* and *S_TBI* deviated from k and S, respectively, which were determined accurately by fitting an asymptote model to the time-series mass of the remaining green and rooibos teas (*k_fitting* and *S_fitting*). Nevertheless, *k_TBI* and *S_TBI*, which can be determined in a cost- and labor-effective way, might be useful for comparative analyses, indicating the relative values of k and S across different soils. Therefore, this study investigated the correlations of *k_TBI* and *S_TBI* with *k_fitting* and *S_fitting*, respectively, to examine if they are positively correlated with each other, in which case these indexes are useful for comparative analyses. Data for the analysis were collected from literature on the time-series mass of both green

and rooibos teas remaining. An incubation study was performed to obtain time-series tea decomposition data under relatively dry conditions.

2. Materials and Methods

2.1. Incubation Study

An incubation study was performed to investigate the correlations of *k_TBI* and *S_TBI* with *k_fitting* and *S_fitting*, respectively, under relatively dry conditions. A surface soil sample (0–10 cm) collected in a Japanese cedar (*Cryptomeria japonica* [L. f.] D. Don) plantation at the Tatsudayama research site in Kumamoto, Japan (32.82° N, 130.73° E) was used for the incubation. The average annual temperature in the study area is 17.1 °C, and the annual precipitation is 1951 mm (Agro-Meteorological Grid Square Data of the National Agriculture and Food Research Organization). The soil sample was sieved through a 4 mm sieve. Large pieces of organic matter were removed from the soil sample. Because the incubation study aimed to obtain data under relatively dry conditions, the soil was pre-incubated to decrease the soil water content. The initial soil water content of 0.39 (*w*/*w*) decreased to 0.31 (*w*/*w*) during the 25 h drying process. After pre-incubation, green (EAN: 8714100770542; Lipton, London, UK) and rooibos (EAN: 8722700188438; Lipton, London, UK) tea bags were buried in 100 g of fresh soil in a polyethylene terephthalate bottle. Although Lipton has changed the bag material from 0.25 mm woven mesh to nonwoven mesh, the tea materials inside were unchanged (see Teatime 4 Science; https://www.teatime4science.org/ (accessed on 1 December 2021)). Therefore, I reproduced woven tea bags using the tea inside the nonwoven tea bags and 0.25 mm of mesh (For detailed information, see Mori et al. (2021) [8]). The teas were oven-dried at 70 °C for >72 h, and the initial mass was recorded before the start of incubation. Five bottles were placed in an incubator at 25 °C for 5, 13, 23, 58, and 90 days. The bottles were covered with polyethylene sheets to prevent water evaporation [9]. At the end of the incubation, the tea bags were immediately placed in an oven and dried at <70 °C to prevent further decomposition. Then, after cleaning the surface soil and removing the bags, the teas were oven dried at 70 °C for >72 h and weighed.

2.2. Determination of k_TBI, S_TBI, k_Fitting, and S_Fitting

k_TBI was calculated following Keuskamp et al. (2013) [2] as follows:

$$k_TBI = (ln(a_r) - ln(a_r - Mloss_R))/t \quad (1)$$

where *Mloss_R* is the mass loss ratio of rooibos tea during an incubation period of t days (generally $t = 90$), and a_r is the unstabilized hydrolysable fraction of rooibos tea determined as follows (because it is assumed that the stabilization factor S of rooibos tea equals that of green tea):

$$a_r = H_r \times (1 - S_TBI) \quad (2)$$

where H_r (0.552) is the hydrolysable fraction of rooibos tea [2], and *S_TBI* is determined as follows:

$$S_TBI = 1 - a_g/H_g \quad (3)$$

where H_g (0.842) is the hydrolysable fraction of rooibos tea [2], and a_g is the unstabilized hydrolysable fraction of green tea. In the framework of the TBI approach, a_g equals the mass loss ratio of green tea during a 90-day incubation because it is assumed that most of the unstabilized hydrolysable fraction of green tea is decomposed within 90 days.

k_fitting and *S_fitting* were calculated following Mori (2022) [7]. *k_fitting* was calculated by fitting the asymptote model to the mass of rooibos tea remaining as follows:

$$Mass\ of\ rooibos\ tea\ remaining = a_r_fitting \times e^{-k_fitting \times t} + (1 - a_r_fitting) \quad (4)$$

where t is the number of days after the start of incubation. The asymptote ($1 - a_r_fitting$) and $k_fitting$ of the model were determined by nonlinear regression ("nls" library). The mass of green tea remaining was fitted by the asymptote model:

$$Mass\ of\ green\ tea\ remaining = a_g_fitting \times e^{-k_fitting_G \times t} + (1 - a_g_fitting) \quad (5)$$

where the asymptote ($1 - a_g_fitting$) and $k_fitting_G$ of the model were determined by nonlinear regression in the same manner. $S_fitting$ was determined by the following equation:

$$S_fitting = 1 - a_g_fitting / H_g \quad (6)$$

2.3. Data Collection from the Literature

Data were collected from published reports of k_TBI, S_TBI, and the time-series mass of green and rooibos teas remaining [2,7,10,11]. Data for aquatic ecosystems [12–14] were not included in this analysis, because the initial leaching of the soluble fraction would change the decomposition curves [13]. Data taken from field experiments [15] were also excluded because environmental conditions such as temperature and moisture were not constant during the incubation. Data using tea bags with nonwoven mesh were included. Different mesh sizes can affect the decomposition pattern of teas by changing the water content inside the tea bags or community composition of decomposers [11]. However, these changes are not problematic in this analysis because those changes influence the k_TBI, S_TBI, and time-series mass loss of green and rooibos teas similarly. To extract data from figures, DataThief 3.0 was used [16]. k_TBI, S_TBI, $k_fitting$, and $S_fitting$ were calculated as described in Section 2.2. If the mass loss data of the teas were not reported on day 90 of an incubation study, the data were estimated using the fitted asymptote model (data from Keuskamp et al. (2013) [2]), or the data from day 91 were used instead (data from Duddigan et al. (2020) [10]). In the present analysis, data collected from the same study area but different subplots or from different incubation studies under different environmental conditions were treated as independent. A linear mixed-effects model with method (TBI vs fitting) as a fixed effect and incubation condition (such as soil type and incubated temperature) as a random effect was used to examine the differences between parameters (S, k, and asymptote) obtained by the TBI approach and those obtained by fitting the asymptote model to the time series data. R ver. 4.0.2 [17] was used for the statistical analysis.

3. Results and Discussion

All of the time-series data on the masses of both green and rooibos tea remaining after decomposition, including the data obtained in the present incubation study (Figures 2a and 3a), were well fitted by the asymptote model (Figures 2 and 3). The fittings of the asymptote model were much better than those of the single exponential model (Figures 2 and 3), which justified the use of the asymptote model rather than the single exponential model for describing the decomposition of teas in the TBI approach. The decomposition curves of rooibos tea were well described by the TBI-based asymptote model in some, but not all, of the incubation studies (Figure 2a,b,j,k,m–o). As Mori (2022) suggested, the TBI-based asymptote model did not well describe the decomposition curve in some soils (Figure 2d–g,i,r), most likely because the essential premise of the TBI approach, i.e., the stabilization factors of rooibos and green teas are equal, was not satisfied [7]. Indeed, the asymptote obtained by the fitting differed significantly from that calculated based on the TBI approach ($p < 0.01$, Figure 4c). The asymptote (i.e., $1 - a_r$) was underestimated by the TBI approach because unstabilized hydrolisable fraction in rooibos tea (i.e., a_r) was overestimated by the TBI approach in most cases (Figure 5a). This unsatisfied assumption of the TBI approach resulted in significant differences between k_TBI and $k_fitting$ ($p < 0.01$, Figure 4a). These results underscore the importance of obtaining time-series mass loss data of teas for accurately determining the early stage decomposition rate k of the asymptote model, as suggested previously [7,18].

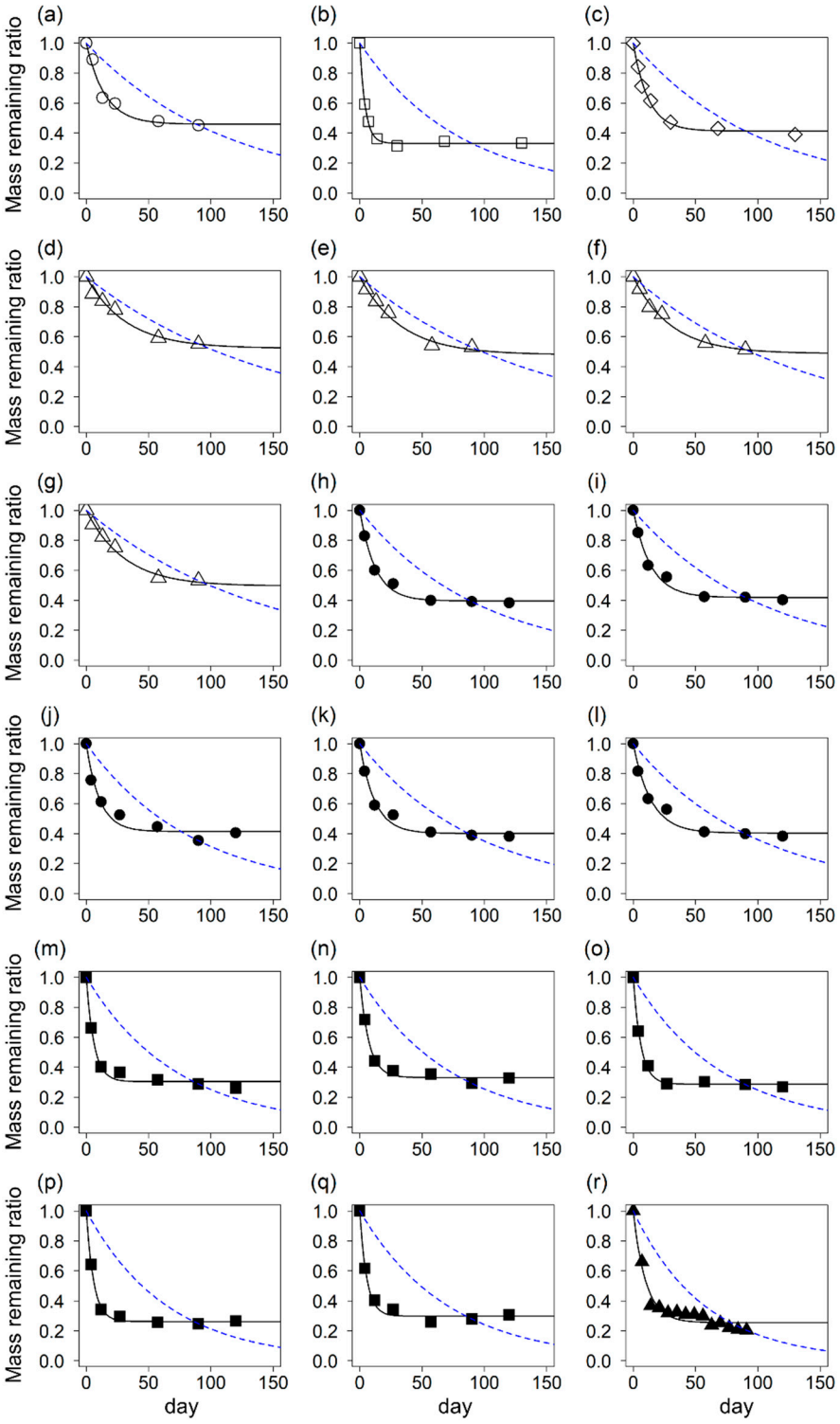


Figure 2. Mass remaining ratios of green tea during the incubation studies. Symbols in the figure indicate the proportion of green tea remaining. Different symbol indicates different incubation condition (such as soil type and incubated temperature). Black lines indicate the fit to the asymptote model. Dashed blue lines indicate the single exponential model describing green tea decomposition. Data were obtained from the present incubation study (**a**), Keuskamp et al. (2013) [2] (**b**,**c**), Mori (2022) [7] (**d**–**g**), Mori (2022) [11] (**h**–**q**), and Duddigan et al. (2020) [10] (**r**). Data points in figures (**b**,**c**,**r**) indicate mean values.

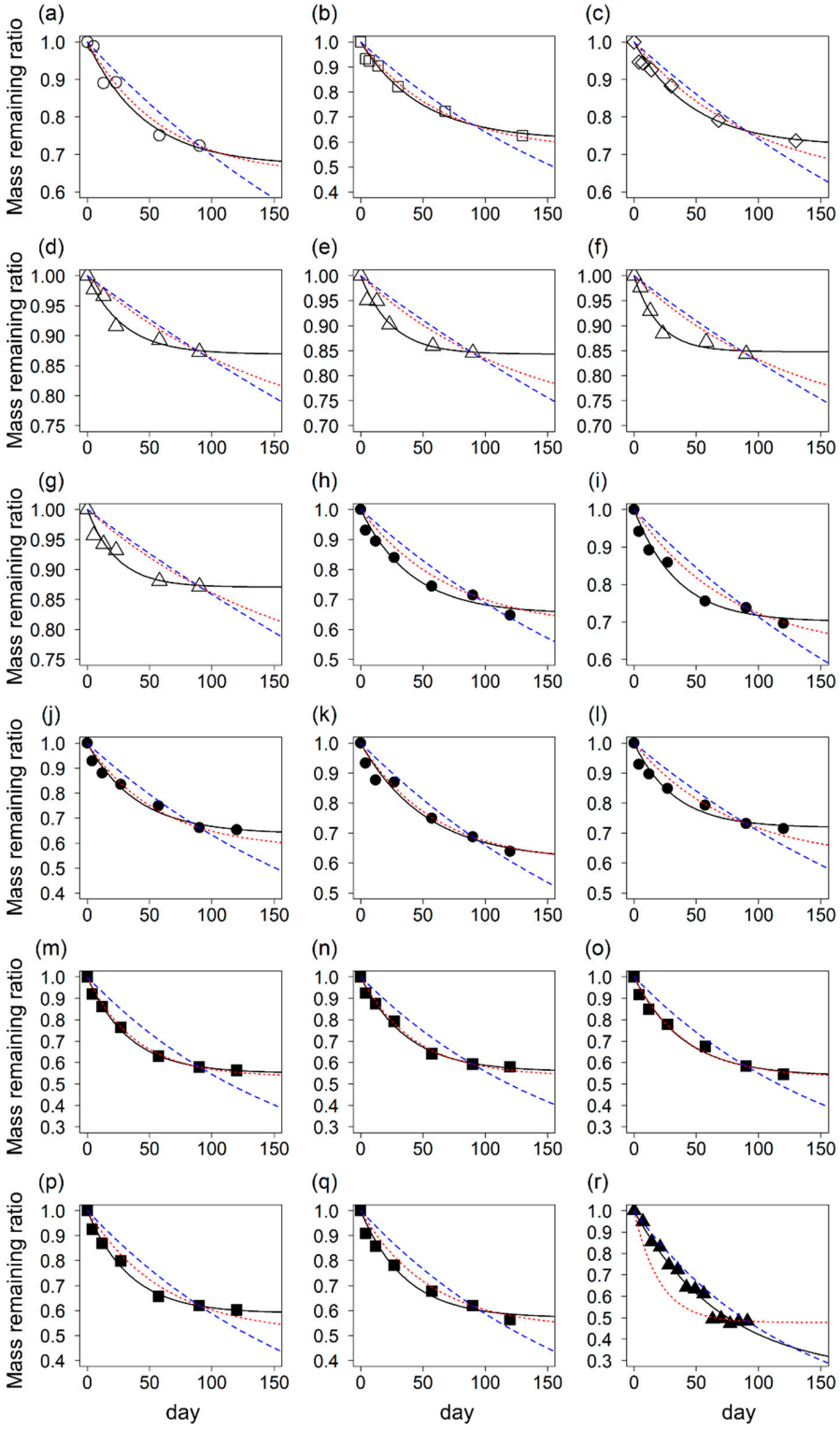


Figure 3. Mass remaining ratio of rooibos tea during incubation studies. Symbols in the figure indicate the proportion of rooibos tea remaining. Different symbol indicates different incubation condition (such as soil type and incubated temperature). Black lines indicate fitting to the asymptote mode. Dotted red lines indicate the asymptote model describing rooibos tea decomposition based on the TBI approach. Dashed blue lines indicate the single exponential model describing rooibos tea decomposition. Data were obtained from the present incubation study (**a**), Keuskamp et al. 2013 (**b**,**c**), Mori 2022a (**d**–**g**), Mori 2022b (**h**–**q**), and Duddigan et al. 2020 (**r**). Data points in figures (**b**,**c**,**r**) indicate mean values.

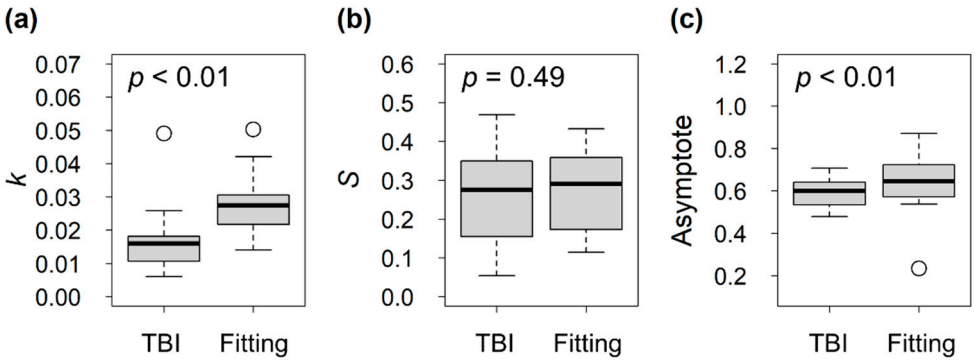


Figure 4. Comparisons of (**a**) k, (**b**) S, and (**c**) asymptote (i.e., $1 - a_r$) obtained by the TBI approach and those obtained by fitting the asymptote model to the time series data. Statistical significances were examined using a linear mixed-effects model.

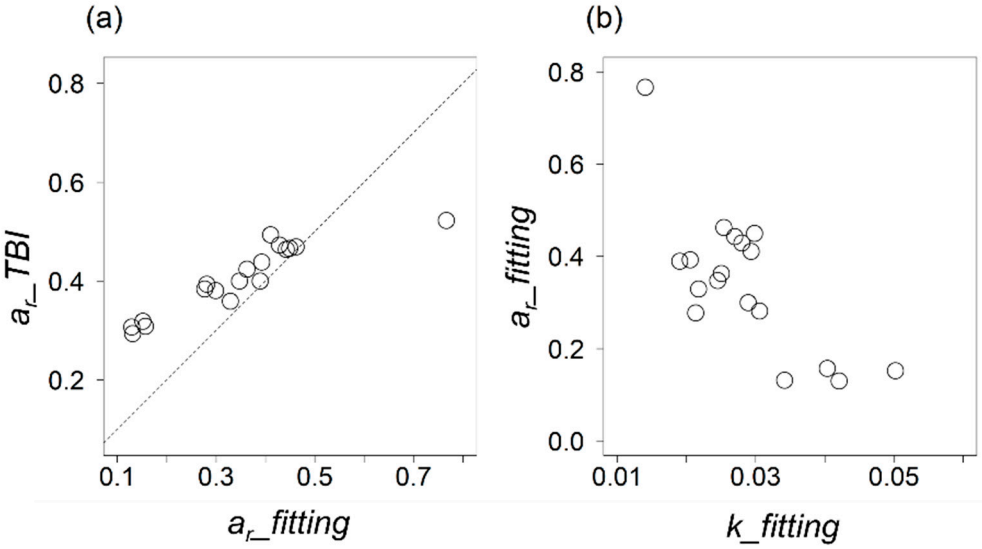


Figure 5. Relationships between (**a**) a_r*_TBI* and a_r*_fitting* and (**b**) a_r*_fitting* and *k_fitting*. Dashed line indicates the 1:1 line.

The purpose of this study was to test if *S_TBI* and *k_TBI* are indicative of the relative values of *S_fitting* and *k_fitting* across different soils. If *S_TBI* and *k_TBI* are positively correlated with *S_fitting* and *k_fitting*, respectively, they can be used as an indicator of the relative decomposition rate across different soils, which would be useful for comparative analyses, although they do not determine k and S accurately. However, the results showed that *k_TBI* and *k_fitting* were not positively correlated but were significantly negatively correlated (Figure 6a). This indicates that *k_TBI* is not indicative of the asymptote model-based decomposition constant k and is therefore not useful for comparative analysis of the decomposition rates of rooibos tea among soils. The negative correlation between *k_TBI* and *k_fitting* was observed probably because of the following mechanism. The overestimation of a_r*_TBI* became greater as a_r*_fitting* decreased (Figure 5a). Because smaller a_r*_fitting* was associated with larger *k_fitting* (Figure 5b), greater overestimation of a_r*_TBI* was associated with larger *k_fitting*. Because overestimation of a_r*_TBI* causes underestimation of *k_TBI* [19], underestimation of *k_TBI* became greater as *k_fitting* became larger, which caused negative correlations between *k_TBI* as *k_fitting*. On the other hand, *S_TBI* and *S_fitting* were significantly positively correlated (Figure 5b), indicating that *S_TBI* can be used as an indicator of S. Furthermore, the regression line was close to the 1:1 line, and the difference between *S_TBI* and *S_fitting* was not significant ($p = 0.49$, Figure 4b). Although a previous study reported that *S_TBI* and *S_fitting* were slightly but significantly different [7], the difference might be negligible. More data are required to examine this.

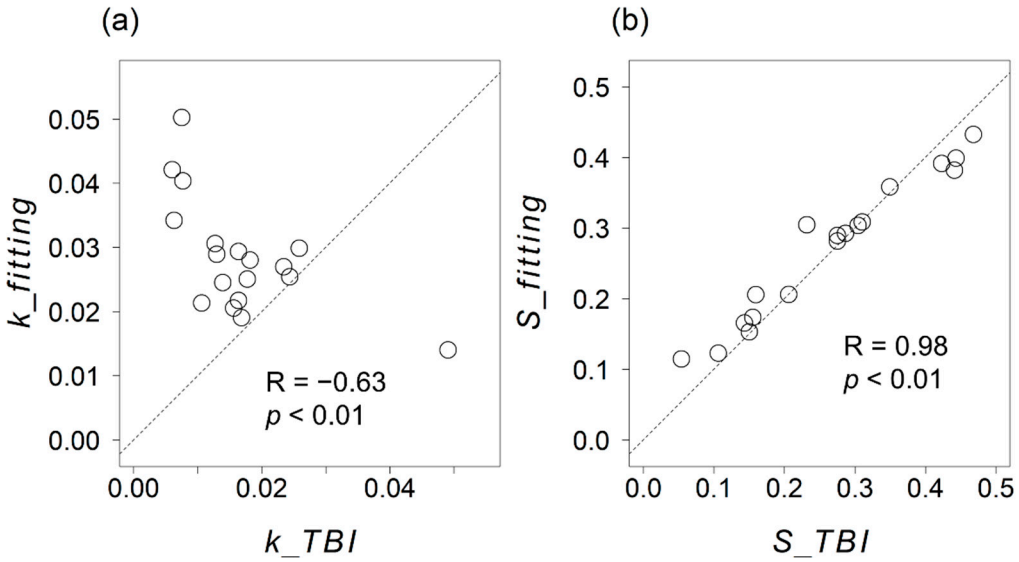


Figure 6. Relationships between (**a**) *k_fitting* and *k_TBI* and (**b**) *S_fitting* and *S_TBI*. Dashed lines indicate the 1:1 lines.

The finding that the *k_TBI* does not indicate the asymptote model-based decomposition constant *k* does not reduce the value of the TBI approach. The tea bags provide effective standardized materials for evaluating organic matter decomposition; the method is cost-effective and reduces the effort needed to prepare litter bags. Because of these advantages, obtaining time-series data is not difficult using the TBI approach, and I recommend obtaining time-series data to determine the early-stage decomposition constant *k* of rooibos tea. Alternatively, to reduce the effort in obtaining decomposition data as much as possible, I recommend using the raw mass loss data of rooibos tea, rather than *k_TBI*, as suggested previously [19], because *k_TBI* is biased by an unsatisfactory assumption of the TBI approach: the stabilization factors of rooibos and green teas are equal.

This study demonstrated that *k_TBI* does not indicate the relative decomposition rate across different soils and is not useful for comparative analyses. However, it might be possible to overcome the unsatisfactory assumption of the TBI approach to improve the *k_TBI* calculation if the asymptote of the rooibos tea decomposition curve can be predicted by the mass loss data of green tea (Figure 5a, note that a_r*_TBI* is the constant factor of a_g*_TBI*). Because the currently obtained data are insufficient to test this, more time-series data need to be obtained from various soil types.

4. Conclusions

The present study examined if *k_TBI* and *S_TBI*, which can be determined in a cost- and labor-effective manner, indicates the relative values of *k_fitting* and *S_fitting* across different soils and be useful for comparative analyses. The correlations of *k_TBI* and *S_TBI* with *k_fitting* and *S_fitting*, respectively, were examined, because if they are positively correlated with each other, these indexes are useful for comparative analyses. However, the result showed that *k_TBI* was negatively correlated with *k_fitting*, indicating that *k_TBI* is not indicative of the asymptote model-based decomposition constant *k* and is therefore not useful for comparative analysis of the decomposition rates of rooibos tea among soils. This study underscores the importance of obtaining time-series data for accurately determining the decomposition constant of an asymptote model describing the decomposition curve of rooibos tea. Nevertheless, *S_TBI* was positively correlated with *S_fitting*, indicating that *S_TBI* can be used as an indicator of *S*.

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Data Availability Statement: Data supporting the reported results can be sent upon request by the corresponding author.

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Conflicts of Interest: The author declares no conflict of interest.

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