Can Nonwoven Tea Bags Be Used to Determine the Tea Bag Index?

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Abstract: Researchers have studied the impact of various anthropogenic activities on litter decomposition rates because of their large impact on the future carbon budget and climate change. However, any assessment of the global-scale impact of anthropogenic activity on litter decomposition requires standardized methods that can exclude the variability of litter chemistry. The Tea Bag Index (TBI) is widely used as a standardized method to obtain both the decomposition constant $k$ of early-stage litter decomposition and the stabilization factor $S$. Recently, a tea bag manufacturer changed the materials and size of the tea bag mesh from a 0.25 mm woven mesh to a nonuniform, nonwoven mesh. To test whether these changes in mesh materials have any effect on the TBI approach, an incubation study was performed. Obtaining time series decomposition data for both green and rooibos teas, two essential assumptions of the TBI approach were examined: (i) that most of the unstabilized hydrolyzable fraction of green tea is decomposed within 90 days (unless the environment is unfavorable for decomposition) and (ii) the $S$ of green tea is equal to that of rooibos tea. The results did not show a clear breakdown of the first assumption of the TBI approach due to the changes in mesh materials, and they did not support the second assumption. The $S$ of rooibos tea determined by fitting an asymptote model to the time series data was significantly larger than the TBI-based $S$. In conclusion, the TBI may be undeterminable using nonwoven tea bags.

Keywords: asymptote model; decomposition constant $k$; nonwoven tea bags; stabilization factor $S$; Tea Bag Index

1. Introduction

Soils are the largest carbon (C) pool in terrestrial ecosystems [1], and litter decomposition plays an essential role in regulating soil C dynamics. Many studies have examined the impact of anthropogenic activities on litter decomposition rates because of their large impacts on the future C budget and climate change [2,3]. However, these impacts are influenced by the variability of litter chemistry, because litter chemistry regulates litter decomposition rates. To assess the global-scale impact of anthropogenic activity on litter decomposition, standardized materials are necessary.

Keuskamp et al. [4] proposed the Tea Bag Index (TBI) approach as a way to obtain both the decomposition constant $k$ of early-stage litter decomposition and the stabilization factor $S$, which indicates the stabilized rates of the hydrolyzable fraction during decomposition. This could be ideal for global-scale comparisons of the impact of anthropogenic activity on litter decomposition due to its standardized materials and protocols. Increasing numbers of studies are using this method to evaluate litter decomposition rates [5–11]. However, in 2017, Lipton, the manufacturer, changed the materials and size of the tea bag mesh from a 0.25 mm woven nylon mesh to a nonuniform polypropylene mesh, which was $<$0.2 mm according to two-dimensional pictures [12]. However, this mesh size evaluation is not comparable with woven mesh because the nonwoven material has a nonuniform three-dimensional structure. Although this change could influence the assumptions required to
calculate the TBI, no study has examined whether nonwoven tea bags can also determine the TBI (Teatime 4 Science; http://www.teatime4science.org/ (accessed on 1 December 2021)).

The TBI approach requires two essential assumptions to calculate the decomposition constant $k$ and stabilization factor $S$ using a single measurement of the mass loss ratios of green and rooibos teas. The first assumption is that most of the unstabilized hydrolyzable fraction of green tea decomposes within 90 days (Figure 1), so the decomposition of the unstabilized hydrolyzable fraction of green tea ($a_g$, Figure 1) can be approximated to the green tea mass loss at approximately 90 days (or more than 90 days in an unfavorable environment for decomposition, as suggested by Keuskamp et al. [4]). The stabilization factor $S$ (part of the TBI) is calculated as the ratio of the stabilized hydrolyzable fraction ($H_g - a_g$) to the total hydrolyzable fraction ($H_g$, which Keuskamp et al. [4] reported to be 0.842), as follows:

$$S = \frac{H_g - a_g}{H_g}$$

where $a_g$ is determined as the green tea mass loss ratio at approximately 90 days.

**Figure 1.** The first key assumption of the Tea Bag Index (TBI) approach and how to determine the stabilization factor $S$. It is assumed that the unstabilized hydrolyzable fraction ($a_g$) is mostly decomposed within 90 days, unless the environment is unfavorable for decomposition, and the field data at day 90 can be approximated to the fraction. The figure is modified from Mori et al. [13].

The second assumption is that the $S$ of green tea equals that of rooibos tea, i.e., the stabilized hydrolyzable fraction of rooibos tea ($H_r - a_r$, Figure 2) can be determined by multiplying $S$ by the total hydrolyzable fraction of rooibos tea ($H_r$, Figure 2), as follows:

$$H_r - a_r = H_r \times S$$  \hspace{1cm} (2)
Figure 2. The second key assumption of the Tea Bag Index (TBI) approach and how to determine the decomposition constant \( k \). It is assumed that the stabilization factor \( S \) (i.e., the ratio of the unstabilized hydrolyzable fraction to the total hydrolyzable fraction) of rooibos tea equals that of green tea. The figure is modified from Mori et al. [13].

The unstabilized fraction of rooibos tea \( (a_r) \) can be determined using the following equation:

\[
a_r = (1 - S) \times H_r
\]

where Keuskamp et al. [4] reported \( H_r = 0.552 \). The decomposition constant \( k \) of rooibos tea, another TBI, is determined by fitting an asymptote model to the field mass of rooibos tea remaining with the following equation:

\[
\text{Mass ratio of rooibos tea remaining} = a_r \times e^{-kt} + (1 - a_r)
\]

where \( t \) is the incubation period (approximately 90 days).

This study tested if the changes in mesh materials of Lipton tea bags have any effects on the accuracy of the TBI. Time series decomposition data for green tea were obtained, and the \( a_g \) and \( S \) of green tea were calculated by fitting an asymptote model to the green tea data (hereafter \( a_g_{\text{fitting}} \) and \( S_{\text{fitting}}_G \), respectively). Then, \( a_g_{\text{fitting}} \) and \( S_{\text{fitting}}_G \) were compared with \( a_g \) and \( S \) as determined by the TBI approach, respectively (hereafter \( a_g_{\text{TBI}} \) and \( S_{\text{TBI}} \), respectively), to test the first assumption of the TBI approach: most of the unstabilized hydrolyzable fraction of green tea is decomposed within 90 days unless the environment is unfavorable for decomposition (i.e., \( a_g_{\text{fitting}} \) equals \( a_g_{\text{TBI}} \)). The \( S \) of rooibos tea was also determined by fitting an asymptote model to the rooibos tea data (hereafter \( S_{\text{fitting}}_R \), and compared with \( S_{\text{TBI}} \) to test the second assumption of the TBI approach: the stabilization factor of green tea equals that of rooibos tea (i.e., \( S_{\text{fitting}}_R \) equals \( S_{\text{TBI}} \)). I also compared the decomposition constant \( k \) of rooibos tea determined by fitting an asymptote model to time series rooibos tea decomposition data (hereafter \( k_{\text{fitting}} \)) with \( k \) determined by the TBI approach (hereafter \( k_{\text{TBI}} \)).

2. Materials and Methods

2.1. Soil Sampling

In June 2021, soil samples for an incubation study were collected in a Japanese cedar (Cryptomeria japonica [L. f.] D. Don) plantation located at the Tatsudayama research site in
Kumamoto, Japan (32.82° N, 130.73° E). Japanese cedar plantations are common in Japan. At the study site, I prepared five randomly selected subplots. Soil samples (0–10 cm) were collected from a 20 × 20 cm area using a shovel. Each soil sample was sieved through a 4 mm mesh after removing large pieces of organic matter. At the research site, the mean annual temperature was 17.1 °C and the precipitation 1951 mm (The Agro-Meteorological Grid Square Data of the National Agriculture and Food Research Organization).

2.2. Tea Bags

The TBI approach requires specified tea bags [4]: Lipton green tea bags (EAN: 8710908903595; Lipton) and Lipton rooibos tea bags (EAN: 8722700188438; Lipton). According to the webpage of the developers of the TBI approach (Teatime 4 Science; http://www.teatime4science.org/ (accessed on 1 December 2021)), Lipton has changed the mesh, but not the tea materials inside. I obtained the tea bags indicated by the link on the web page of the Teatime 4 Science site (i.e., EAN: 8714100770542 for green tea and EAN: 8722700188438 for rooibos tea). The chemical properties of the teas have been reported in [4,14].

2.3. Incubation

Fresh soil (100 g) and one green tea bag and one rooibos tea bag were placed in a polyethylene terephthalate bottle (200 mm in height × 60 mm in diameter). The green and rooibos tea bags were placed in the bottle close to each other, which might have influenced the results. In total, 60 bottles (five subplots × two temperature conditions × six time series) were prepared. The soil water content was adjusted by adding deionized water to 45% (w/w), aiming at the ideal water content for decomposition in soil obtained from the same study site [12]. The bottles were incubated for 120 days at 14 or 25 °C (the two temperature conditions). The 120-day incubation period was chosen because fitting-based S determination should use data taken before degradation of the acid-insoluble fraction influences the mass loss (see Figure 2). All bottles were covered with a polyethylene sheet to prevent water evaporation [15]. Tea bags were retrieved 4, 12, 27, 57.25, 90, and 120 days after the start of incubation (six time series). After retrieval, the tea bags were immediately oven-dried for a few days to prevent further decomposition. The dry weight of the tea in the bags was determined by once again placing the teas in an oven (70 °C for >72 h), after removing the tea bag mesh and soil on the surface of the mesh.

2.4. Calculation and Statistics

All statistical analyses were performed using R ver. 4.1.1 [16]. The TBI was calculated following Keuskamp et al. [4] (see Equations (1)–(4)). The obtained time series decomposition data for green and rooibos teas were fitted by asymptote models to determine \( a_g \) and \( k_{\text{fitting}} \), as follows:

\[
\text{Mass ratio of green tea remaining} = a_g \times e^{-k_{\text{green}} \times t} + (1 - a_g) \quad (5)
\]

\[
\text{Mass ratio of rooibos tea remaining} = a_r \times e^{-k_{\text{fitting}} \times t} + (1 - a_r) \quad (6)
\]

where \( k_{\text{green}} \) is the decomposition constant of green tea determined by fitting an asymptote model to time series green tea decomposition data, and \( a_r \) is the unstabilized hydrolyzable fraction of rooibos tea determined by fitting an asymptote model to time series rooibos tea decomposition data. Nonlinear regression (using the “nls” function) was used for the fitting. \( S_{\text{fitting G}} \) was determined in a similar way to Equation (1), as follows:

\[
S_{\text{fitting G}} = (H_g - a_g) / H_g \quad (7)
\]

where \( H_g \), the hydrolyzable fraction of green tea, is 0.842 [4]. \( S_{\text{fitting R}} \) was determined similarly:

\[
S_{\text{fitting R}} = (H_r - a_r) / H_r \quad (8)
\]
where $H_r$, the hydrolyzable fraction of rooibos tea, is 0.552 [4]. I compared $a_g\_TBI$ with $a_g\_fitting$, $S\_TBI$ with $S\_fitting\_G$ and $S\_fitting\_R$, and $k\_TBI$ with $k\_fitting$, using a linear mixed-effect analysis.

3. Results and Discussion

The asymptote model fit the time series data of green tea decomposition well (Figure S1). In most of the subplots, green tea decomposition plateaued 50–60 days after the start of incubation (Figure S1), implying that the first assumption of the TBI approach holds true. At 14 °C, $a_g\_TBI$ and $a_g\_fitting$ were not statistically different ($p = 0.22$, Figure 3a). Although $a_g\_TBI$ and $a_g\_fitting$ were significantly different at 25 °C ($p < 0.05$, Figure 3b), the difference was small ($a_g\_TBI$ and $a_g\_fitting$ was 0.61 and 0.59, respectively). The results of the comparison of $S\_fitting\_G$ and $S\_TBI$ were the same as those of $a_g\_TBI$ and $a_g\_fitting$ because S is a linear function of $a_g$ (see Equation (1)). Thus, the results did not show a clear breakdown of the first assumption of the TBI approach due to the changes in mesh materials. This is consistent with Mori et al. [12], who reported that the change in mesh materials from woven to nonwoven tea bags did not change the mass loss ratio of green tea bags during a 90-day field incubation.

![Figure 3. Comparison of the unstabilized hydrolyzable fraction of green tea ($a_g$) determined by fitting an asymptote model to time series data of green tea decomposition ($a_g\_fitting$) and $a_g$ determined by the TBI approach ($a_g\_TBI$) at (a) 14 °C and (b) 25 °C, and stabilization factor $S$ of green tea determined by fitting an asymptote model to time series data of green tea decomposition ($S\_fitting\_G$) and $S$ of green tea determined by the TBI approach ($S\_TBI$) at (c) 14 °C and (d) 25 °C. * $p < 0.05$.](image-url)

However, the second assumption was not supported in the incubation study. $S\_TBI$ was significantly smaller than $S\_fitting\_R$ (Figure 4a,b), indicating that the $S$ of rooibos tea
was underestimated in the TBI approach in this study. Accordingly, the decomposition constant $k$ was also underestimated (Figure 4c,d), because $k$ is dependent on $S$ [17]. The present study indicates that the stabilization factor $S$ of rooibos tea is underestimated if nonwoven tea bags are used instead of woven tea bags, which also influences the determination of $k$.

![Figure 4](image-url)

**Figure 4.** Comparison of the stabilization factor of rooibos tea determined by fitting an asymptote model to time series data of rooibos tea decomposition ($S_{\text{fitting \_R}}$, see Equation (8)) and by the TBI approach ($S_{\text{TBI}}$, determined based on the mass loss of green tea, assuming that $S$ of rooibos tea equals that of green tea) at (a) 14 °C and (b) 25 °C. The decomposition constant $k$ of rooibos tea determined by fitting an asymptote model to time series data of rooibos tea decomposition ($k_{\text{fitting}}$, see Equation (6)) and by the TBI approach ($k_{\text{TBI}}$) at (c) 14 °C and (d) 25 °C. * $p < 0.05$.

It was not clear what caused the differences between $S_{\text{fitting \_R}}$ and $S_{\text{TBI}}$ and inaccurate TBI determination with the nonwoven tea bags. Changes in the mesh materials could influence the decomposition pattern of both teas via several mechanisms. First, mesh size could have altered the decomposition pattern through a change in the water content inside the tea bags. Second, the community composition of decomposers involved in tea decomposition could also have changed, influencing the decomposition pattern. Third, the nonwoven mesh itself might have contained nutrients that could have altered the decomposition pattern. However, these changes in decomposition pattern could not explain the inaccurate TBI determination with nonwoven tea bags because the environmental changes caused by the mesh change affected the green and rooibos teas equally. In other words, at least under the same environmental conditions provided by the changes in mesh materials (i.e., altered water content, shift in decomposer community, or nutrient enrichment), even woven tea bags cannot accurately determine the TBI. It is possible that...
the TBI determination was inaccurate because the green and rooibos teas were placed close to each other in the present incubation study. Rooibos tea decomposition could have been accelerated by the labile C or nutrient-rich dissolved organic matter derived from green tea. However, in this case, $S_{fitting\_R}$ is likely to be smaller than $S_{TBI}$, which is opposite to the results of this study.

It might also be possible that the difference between $S_{TBI}$ and $S_{fitting\_R}$ would be observed even in the original methodology using woven bags. Note that the second assumption of the TBI approach (i.e., the $S$ of rooibos tea equals that of green tea) has never been verified, despite the widespread use of the TBI approach. Mori et al. [13] reported that this assumption was not supported in a forest because a contradictory result was observed under the assumption that the amount of rooibos tea decomposed exceeded the decomposable fraction of rooibos tea. Several researchers observed similar results [5,8]. Thus, the second assumption of the TBI approach may not always be true, even in the original methodology. This is reasonable because the chemical compositions of green and rooibos teas differ substantially, as Duddigan et al. [14] reported. Future work needs to validate this essential assumption for the TBI approach using woven mesh bags.

Overall, this study demonstrates that the TBI may be undeterminable using nonwoven tea bags. However, because this study used soil samples taken from a single study site, more observations are necessary to obtain a definitive conclusion. Moreover, the mesh materials were recently changed to plant-based materials (Teatime 4 Science; http://www.teatime4science.org/ (accessed on 1 December 2021)). The validity of the TBI approach using the new tea bags with a plant-based mesh should be tested.

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**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ecologies3020014/s1, Figure S1: Time-series decomposition data of green tea. Each line indicates an asymptote model fitted to the data. Each red dotted line indicates the fitting-based asymptote; Figure S2: Time-series decomposition data of rooibos tea. Each black line indicates an asymptote model fitted to the data. Each red line indicates the asymptote model determined by the TBI approach.

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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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