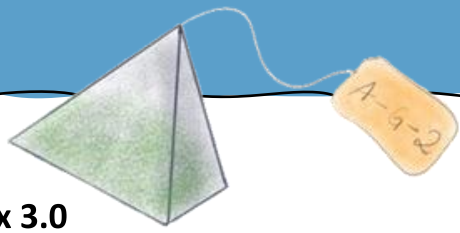

# Application manual for the Tea Bag Index 3.0

**attributable to the article:**

## "New directions for the Tea Bag Index - alternative teabags and concepts can advance citizen science"

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### Theoretical background

In 2013, the team of authors Keuskamp et al. published the study "Tea Bag Index: a novel approach to collect uniform decomposition data across ecosystems". A breakthrough idea: we can better understand the soil microbial activity of ecosystems using tea bags buried in the soil for 90 days! This approach has been applied in many countries and even more studies. Unfortunately, the tea bags required for the approach were no longer commercially available after only a few years. The team behind "Teatime4science" therefore came up with the idea to study the new tea bags produced by the same manufacturer, thus turning the original **T**ea **B**ag **I**ndex into TBI 2.0 (http://www.teatime4science.org/about/the-project/). We showed in our study "New directions for the Tea Bag Index - alternative teabags and concepts can advance citizen science" that new ideas are needed to further develop the quality and applicability of the TBI. For this purpose, not only different tea varieties but also novel approaches to calculate the TBI should be tested experimentally. This manual describes one possible direction which we call the TBI 3.0. We invite you to use our toolkit to conduct your own study and welcome you to send your results and experiences to the mail address above!

### Target parameters of the TBI 3.0

The TBI simplifies the decomposition behavior of a tea substrate in the first months by assuming an asymptote model (purple line in Figure 1). Although this is a gross simplification, the function is very good for modeling decomposition. But first let us look at the decomposition curve of an example tea substrate. Once the tea bag is buried in the soil (t = 0), it is exposed to degradation by (micro)organisms. At the beginning, a particularly large amount of mass is lost, since there is a lot of labile substrate. This can be colonized and decomposed by bacteria and fungi, for example. The more time passes, the less additional substrate is decomposed by the organisms. A site-specific, non-labile fraction remains, which is decomposed much more slowly. Environmental factors (e.g. soil moisture) at the study site determine the velocity of mass loss.

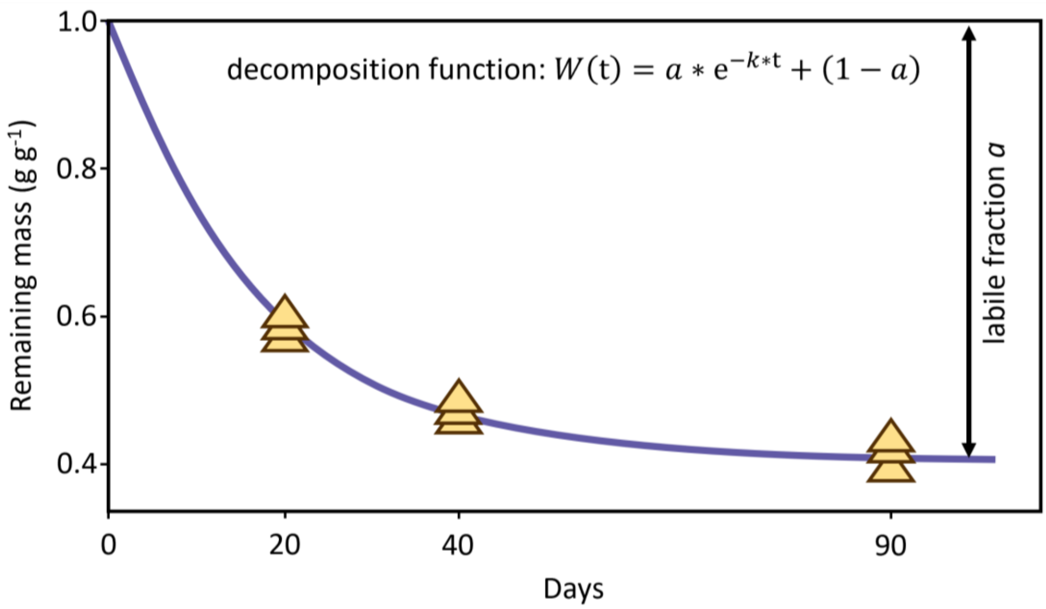
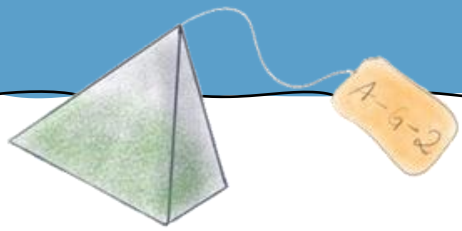



Figure 1: Schematic illustration of the TBI 3.0. The purple curve represents the characteristic decomposition behavior of an easily decomposable tea substrate. Its decomposition function is shaped by *a* and *k* (e stands only for the number 2.718... and t for days).



As you can see in the equation above ("decomposition function"), there are two parameters that make up the TBI 3.0: the decomposition constant $k$ ($d^{-1}$) and the labile fraction $a$ ($g\ g^{-1}$). The letter $k$ describes the decomposition progress with time, i.e., the rate at which the microbes metabolize the substrate. By decomposing labile organics, the microbes recover the built-in nutrients. Later, when microbes die or release the nutrients to the soil solution, they become plant-available again. The variable $a$ points to the saturation level of the curve. The remaining mass ($W(t)$ at t = 90 or higher) is the fraction that is harder to decompose, i.e., that is stabilized because of chemical or physical reasons. In a nutshell, **the higher $k$ is, the higher the microbial activity at the study site. The larger $a$ is, the more organic material is decomposable.**

## How to plan your own study with the TBI 3.0

Each tea variety has unique parameters $k$ and $a$ at each individual location. In the end, our goal is to understand how (diverse) labile organic substances are decomposed in general. For this purpose, it is advisable to try as many different types of tea as possible. In the data entry table provided to you ("data_input_TBI3.0.xlsx"), there is space for six different varieties of tea (or different products of the same variety). We would not recommend rooibos tea, as it decomposes too slowly to be well researched in three months. Of course, you can investigate your ecological research question: You have space for six different sites that you want to compare. Using the results of the TBI, you can then analyze them in terms of their decomposition rate ($k$) and decomposability ($a$) of labile organic matter. In the Excel file you also have space to briefly describe your experiment. In general, you should only fill in the green cells in the first sheet of the data entry table. The other sheets already show first results of the remaining masses ($W(t)$). You should plan to visit your experimental sites after about 20, 40 and 90 days to dig out part of the tea bags again.

## How to handle your samples

1. You will have three incubation intervals (preferably 0-20 days, 0-40 days and 0-90 days) and will need multiple replicates for each tea variety at each site. We recommend that you work with triplicates. So, for each location you need 9 equivalent tea bags of the same product (three for each interval). Enter all the names for your tea bags and locations in "data_input_TBI3.0.xlsx" in the sheet "input".
2. Make sure that all tea bags are dry at the beginning. If necessary, you should dry them in a boiler room, an oven or preferably a drying chamber at approx. 40 °C. Do this until the weight of the bags remains constant and no more water evaporates.
3. Weigh each tea bag with weave, thread and label it so that you can always identify it later (step 1 in the Excel sheet). Enter the weights in the table at step 2.
4. Open three additional replicates of each of your selected tea products to weigh weave, thread and label (without the substrate). Enter the weights at step 3.
5. Bury all tea bags at the different sites in 8 cm depth. Make sure that the individual tea bags are at least 20 cm apart and that the labels you wrote on them remain recognizable. If you bury the tea bags in a grid and write down how you (randomly) distributed them, you can easily assign them again later.
6. We recommend you to document your study during the incubation period. The more you know about the incubation period (temperature, precipitation, soil parameters), the better you can understand your data. What our toolkit needs to "understand" your data is how many days each interval lasted. Enter the number of days each interval lasted in step 4.
7. After each interval, dig out three replicates of each tea product at every site. Handle each recovered tea bag as follows: Carefully remove all soil particles and roots. Dry the tea bag at up to 70 °C until it no longer loses weight (for 48 hours to be safe). Open the tea bag and weigh only the residual substrate (without weave, thread and label) and record the weight at step 5.

## How to process your data

1. In the sheets W(t)_siteA to W(t)_siteF you can see the calculated remaining masses, which is a first overview of the decomposition dynamics of the tea substrates. Save "data_input_TBI3.0.xlsx" in the same folder as the file "calculation_TBI3.0.R".
2. Open the script "calculation_TBI3.0.R" with the program R Studio. If you don't have it installed yet, download the programs R and R Studio here for free https://posit.co/download/rstudio-desktop/.

If you are using R on your computer for the first time, you will need to install 5 packages that the program will require. We have already provided access to them: You just have to remove the # in lines 1 to 5 of the script in the upper left corner, click in the respective line and press Ctrl+Enter. Do this one after the other for the five new packages in your R script. It might take a few seconds for each package to install, and you get a notification in the lower left window (console) when the installation was successful.

3. Execute the whole script at once by pressing the following key combination: Ctrl+Shift+Enter
   The parameters are then calculated automatically. For a brief overview: The majority of the script is dedicated to data structuring and two loops. The latter ensure that a calculation can be run repeatedly for each individual sample. This central calculation is the "nls()" command in lines 41-46. The "Nonlinear Least Squares" algorithm finds the best possible values for *a* and *k* to approximate the determined W(t) values with the given function (Figure 1). If you have any questions, feel free to write us.
4. You can close R Studio now and find the files "results_TBI3.0.xlsx" and "results_TBI3.0.png" with the results of your study in your folder!

### How to interpret your results

The graph will show your experimental sites' *k* and *a* values. In this example (Figure 2), two different sites were explored, each with two tea varieties. The two tea types differ here in that green, relative to black tea, I) decomposes faster (higher *k*) and II) has a larger labile fraction (higher *a*). Comparing the two sites, the decomposition behavior differs: At Site 2, the organics are converted more slowly (lower *k*) but are decomposable to a larger degree (higher *a*). This difference between the two sites can be demonstrated with both tea types, although the strength of the effects may be different.

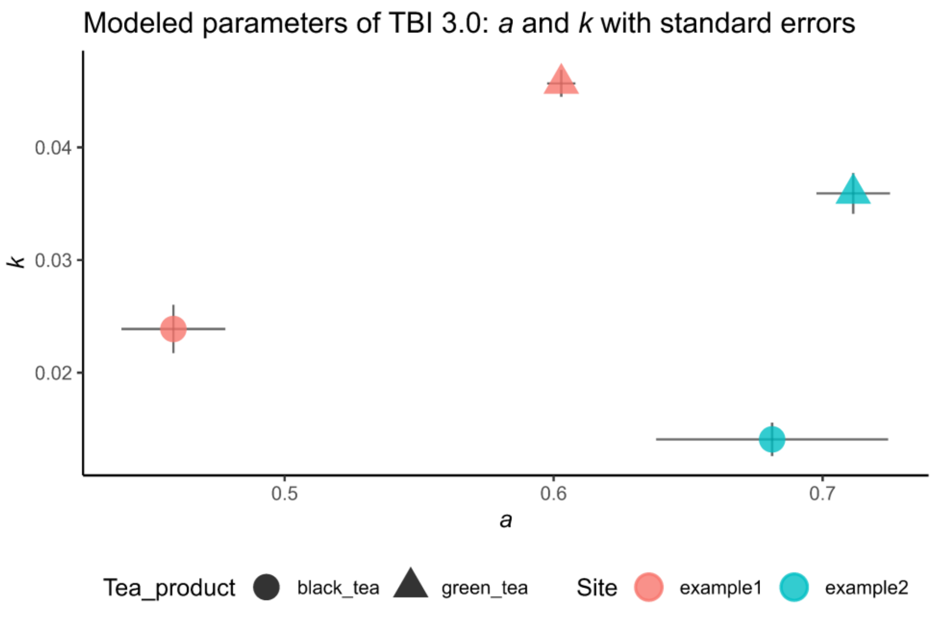



Figure 2: Example result graph of the TBI 3.0. The unit of *a* is g $g^{-1}$ and of *k* is $d^{-1}$, i.e. a measure of the rate of decomposition progress. The grey bars show the standard errors of the modeled parameters. The resulting crosses thus represent the range of values within which the respective combination of a *k* and *a* value can be estimated with greater certainty.

One possible reason for the lower decomposition constants (*k*) at Site 2 could be a generally lower microbial activity at that location. For example, if less litter is supplied there to the soil, or if the aeration is limited, or the soil was previously cold, the microbiome is not developed to directly decompose the added tea substrate as quickly. One explanation for the destabilization of the organics at Site 2 relative to Site 1 (derived from *a*) could be that water scarcity played a greater role at Site 1, causing the decomposability of too-dry organics to be more limited than at Site 2. Since many other reasons for both effects are possible, additional research would be helpful to deepen this example study.

**We are looking forward to your experience reports, reviews and results.**
**Let's work together to push the Tea Bag Index forward.**