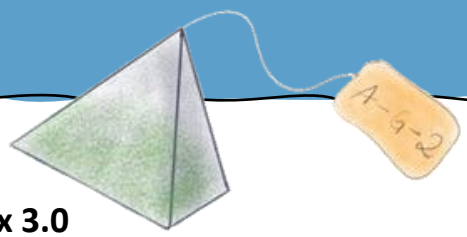


Application manual for the Tea Bag Index 3.0

attributable to the article:

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

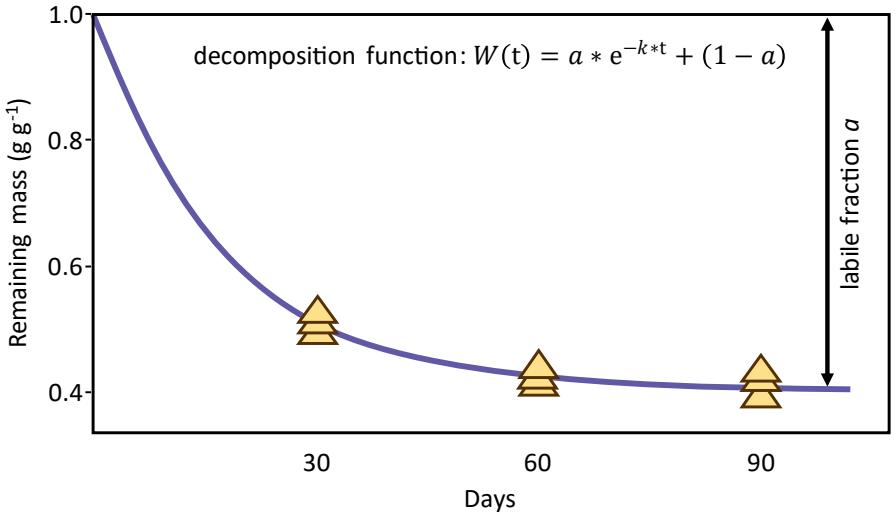
Authors: Thomas Middelanis[a*], Chiara Marie Pohl[a], Dana Looschelders[a], Ute Hamer[a]
[a]Institute of Landscape Ecology, University of Münster, Heisenbergstraße 2, 48149 Münster, Germany
*Corresponding author: thomasmid@posteo.de

Theoretical background

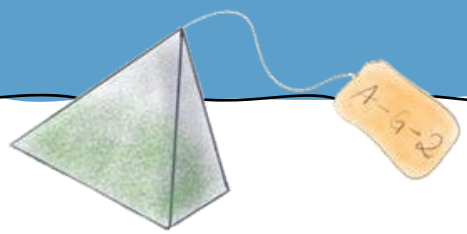
In 2013, the team of authors Keuskamp et al. published the study "Tea Bag Index: a novel approach to collect uniform decomposition data across ecosystems". A breakthrough idea: using tea bags buried in the soil for 90 days, we can better understand the soil microbial activity of ecosystems! This approach has been applied in many countries and much more studies. Unfortunately, after only a few years, the tea bags required to do this were no longer commercially available. Therefore, the team behind "Teatime4science" 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, we call it the TBI 3.0. We invite you to use our toolkit to conduct your own study and welcome your results and experiences to the mail address above!

Target parameters of the TBI 3.0

The TBI assumes the simplification that the decomposition of a tea substrate in the first months behaves like an asymptote model. Although this is a gross simplification, the function is very good for modeling decomposition. You can check this for yourself later on your own data using the error bars (standard errors) in Figure "results_TBI3.0.png". But first let us look at the decomposition dynamics of the tea substrates:



As you can see, there are two parameters that form the TBI 3.0: the decomposition constant k (d^{-1}) and the labile fraction a ($g\ g^{-1}$). The first describes the decomposition progress with time, while the second is where the curve saturates: The remaining mass ($W(t)$ at $t = 90$ or higher) is the fraction that is harder to decompose, i.e., 1 minus a. In a nutshell, **the higher k is, the higher the microbial activity at the study site. Labile organics decompose quicker and nutrients are recovered faster. The larger a is, the more organic material is decomposable. Environmental influences (e.g. soil moisture) at the study site then tend to destabilize organic matter that is more difficult to decompose and to make it accessible to the microbes.**



How to plan your own study with the TBI 3.0

Each tea variety has its own parameters *k* and *a* at each individual location. In the end, our statement should not be how effectively only green tea or only black tea is decomposed in the examined soil, but rather, if possible, how 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 recommend rooibos tea less, as it decomposes too slowly to be well researched in three months. Of course, you also want to be able to 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. If you like, you can send us this file so that we can learn from your experiences. 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 30, 60 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 0-30 days, 0-60 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".
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). 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. 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 (step 4).
6. 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 it 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.
3. Execute the whole script at once by pressing the following key combination: Ctrl+Shift+Enter
4. You can close R Studio now and find in your folder the files "results_TBI3.0.xlsx" and "results_TBI3.0.png" with the results of your study!

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

thomasmid@posteo.de | Institute of Landscape Ecology, University of Münster, Heisenbergstraße 2, 48149 Münster, Germany