

TEA-BAG Addon for the season 2024 / early 2025

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AIM

Research suggests that aboveground interactions with plants are interconnected with belowground soil decomposition. However, the impacts of aboveground plant consumers on soil decomposition are poorly studied. The Tea-Bag Index is a standardised method to quantify decomposition using teabags. Tea mass loss is measured after the tea has been buried in the soil for 3 months. By using this method in the BugNet experimental plots we will evaluate how effects of aboveground insects, molluscs, and fungi on soil decomposition rates vary across different regions, ecosystems, and climates.

If you are interested in doing the teabag addon:

please send an email to Anne Kempel: anne.kempel@slf.ch stating:

- Your name
- Your shipping address
- The number of sites and plots at which you want to implement the teabag addon (usually 1 site with 24 plots).

Calculation will be made to send you the right number of teabags.

METHODS

Overall understanding

Decomposition will be estimated in a standardised way measuring the mass loss of 2 different kinds of tea. A green tea (Lipton Green tea: EAN 87 10908 90359 5 or EAN 8722700 05552 5) and a rooibos tea (Lipton Rooibos tea: EAN 87 22700 18843 8) will be sent to you for the experiment.

The green tea is used as a representative foliage litter that is nutrient-rich, while the rooibos tea represents recalcitrant litter that is nutrient-poor. The tea bags will first be weighed, then they will be buried in the soil for **3 months** and then collected and weighed again. **3 teabags of each kind** will be buried in each plot, which is a total of **6 teabags per plot**. Each teabag will be separated **15 cm from the others** and put at a depth of **8 cm beneath the soil surface**.

While the teabags are buried, temperature (and moisture if possible) should be recorded. Information about the soil (C, N and P) should also be added, if this has not already been done for the “experimental part” of the BugNet project. If not done, please contact Anne Kempel (anne.kempel@slf.ch) and take some soil sample according to the experimental protocol.

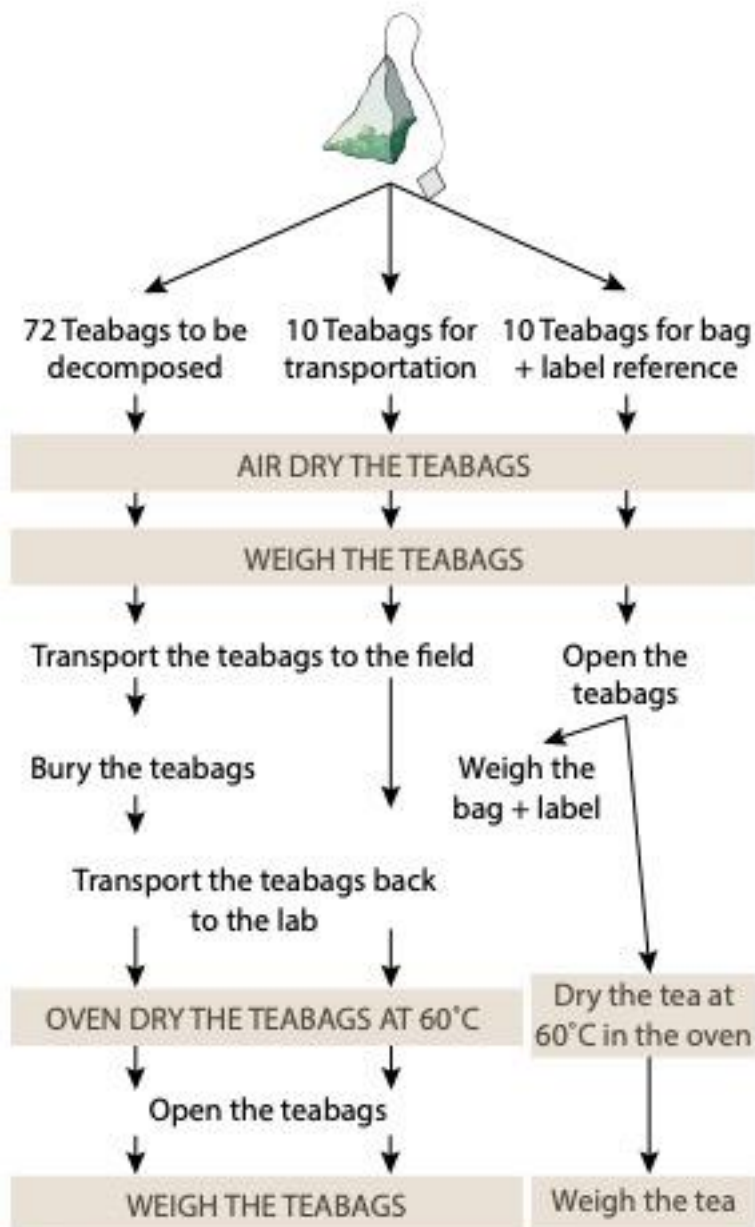


Figure 1: Overview of the protocol

Before burying teabags

Label all teabags, e.g. with site_ID, block_ID, plot_ID and teabag ID or with a running number according to the teabag-addon spreadsheet. For example: S-G-1-8-5 and S-R-1-8-5, with S the site_ID, R or G the tea type (R for rooibos and G for green), 1 the block_ID, 8 the plot_ID and 5 the tea_ID. The label should be written with a **permanent marker**. Label 10 additional teabags of each tea type, numbered from LG-1 to LG-10 and LR-1 to LR-10, where L indicates Lab (they will not be buried in the field), and 10 additional teabags of each tea type numbered from TG-1 to TG 10 and TR 1- TR10, where T indicates transportation.

After the labelling phase, all teabags will be **weighed before being buried** in the soil. First **air dry the teabags** (e.g., 1-2 days in your office). Then use a 0.001 g precision scale and enter all weights in the teabag addon excel file. The weight should take into account the whole teabag, with the tea, the bag, string and the label; Figure 1.

The 10 extra bags (L) are used to estimate the average weight of the bag, string and the label, to give an estimate of the tea weight in each bag. Process as follow:

Air dry the first 10 additional teabags (LG-1 to LG-10 and LR-1 to LR-10) and weigh them. Then open them, remove the tea, weight the airdry tea and place it in a labelled paper bag. Also weigh the teabag alone (without the string and the label), the string alone and the label alone. Then dry the tea at 70°C, tea that is now in a paper bag, and weigh it; Figure 1.

Burying the teabags

At the beginning of the growing season, bury 6 teabags (3 green tea and 3 rooibos tea) in each plot as indicated in Figure 2, as an example. If you have a site-specific addon and the position indicated in Figure 2 is not suitable for your site, you can also find a different location, ideally not at the edge of the site. Mark down exactly where your tea bags are buried and make a map if this will help you to relocate the bags.

Put the teabags 8 cm deep into the ground and separate them at least 15 cm from each other; Figure 2. The label should be on the surface, to be able to find the bags later on.

The last 10 additional tea bags (from TG-1 to TG-10 and TR-1 to TR-10) will not be buried and brought back to the lab and keep there. They are used to see the potential impacts of transportation on the tea weight.

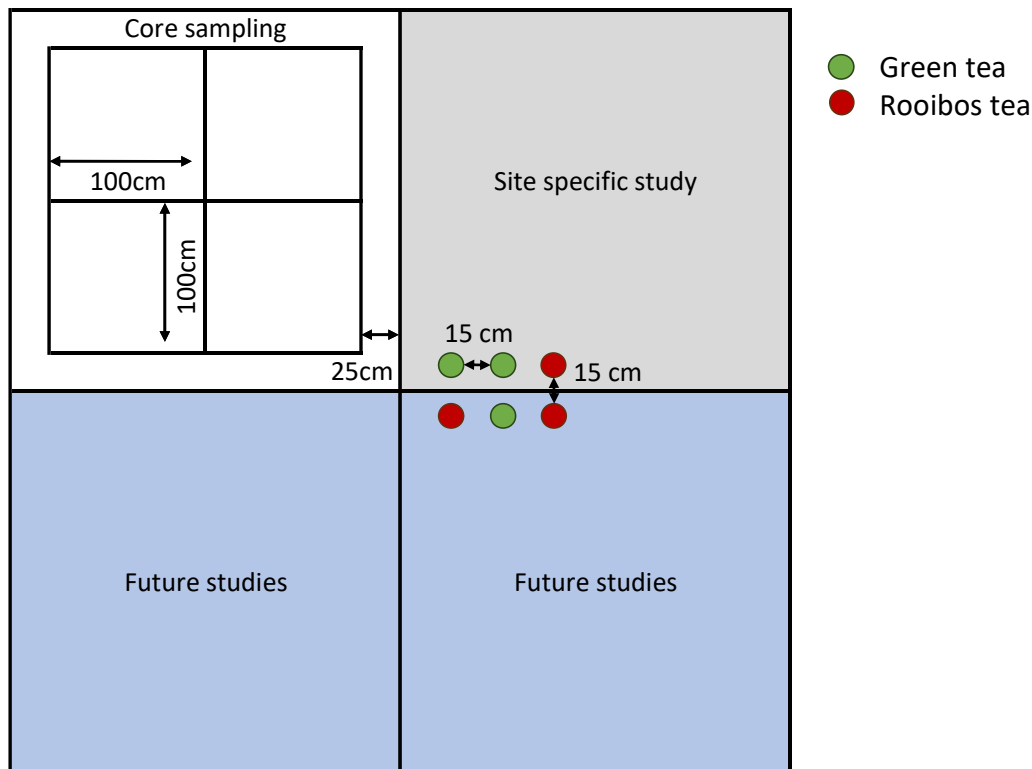


Figure 2: Position of tea bags in the BugNet plots

Use a hand shovel to gently put the teabags in the ground, as presented in Figure 3.

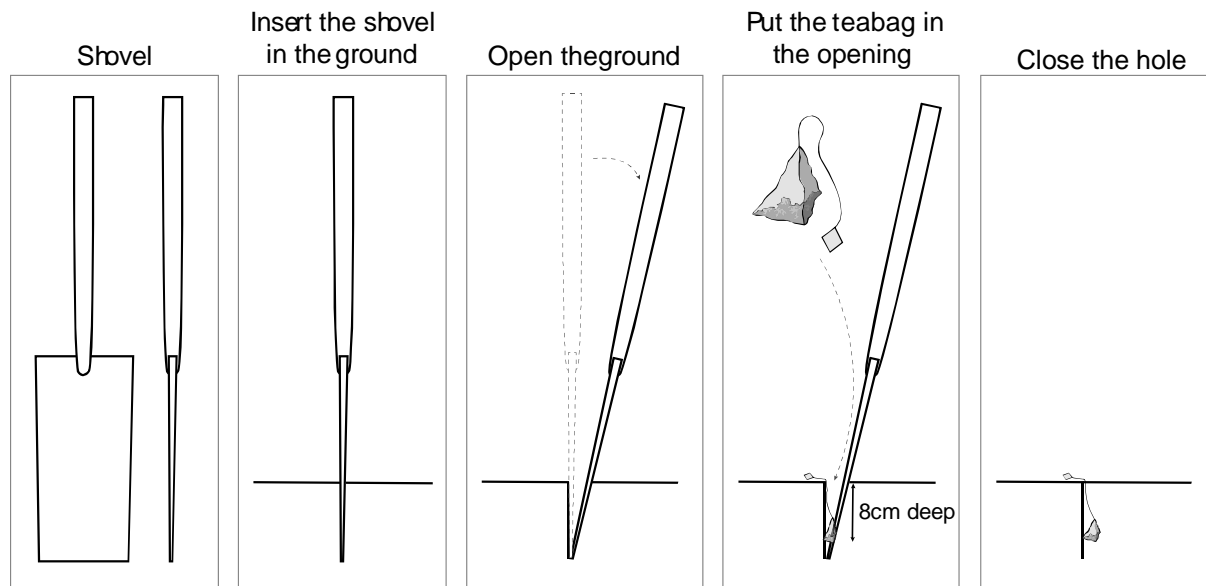


Figure 3: Burying process

Temperature and moisture measurement (optional):

If possible, record the soil temperature (and if possible, the soil moisture) during the 3 months the teabags are buried.

- If you have enough temperature loggers, then place a logger in each plot at the same time the teabags are being buried.
- If you don't have enough temperature loggers, use one logger to capture temperature and moisture in the middle of the site as a reference. If you have 8 additional loggers, place them in each plot of block 1. After two weeks, remove them and place them in the plots of block 2. After another two months remove them and place them in the plots of block 3 and so on. This gives us the opportunity to calculate plot-specific variation in temperature/moisture. If you have only a few loggers, then try to change the location of the loggers after shorter period of times, so that each plot has been assessed at least for a short period.
- If you don't have any loggers, you can still participate in the tea-bag decomposition addon. Bury the teabags and weigh them after 3 months and send us temperature data from the nearest meteorological station.

Collection of the teabags

After **3 months (90 days)** you should collect the teabags. Please record the date when buried and the date when collected and indicate it in the excel file. Also bring the 10 teabags from TG-1 to TG-10 and TR-1 to TR-10 with you, which are used to test for any effects of transportation.

After collection of the teabags

In the lab, open each teabag (collected in the field and the 10 additional ones just transported; TG-1 to TG-10 and TR-1 to TR-10) to weight the tea content as follows; Figure 4:

- **Dry the teabags for 48h at 70° C**
- Clean the entire teabag with a painting brush to remove the soil and other elements, if needed.
- Define the state of the teabag and put it in the excel file:

- **0** -> the teabag is intact, has no holes, no mycelium, no root.
 - **1** -> the teabag has
 - several small holes (<3 mm) or few medium holes (between 4 and 6 mm), or
 - 1 or 2 roots, or
 - less than 10% mycelium contamination
 - **2** -> the teabag has a hole, roots or mycelium that impact the weight of the tea.
 - **3** -> the teabag may have lost its content because of holes > 7 mm or several medium holes (between 4 and 6mm).
 - **4** -> the teabag was not found.
- Open the teabag with scissors on 2 sides from the top. Empty the bag above a clean sheet of paper. Turn the corners inside out and brush the inside of the teabag to make sure to remove all the tea materials from the bag.
 - Remove all roots and remaining parts of teabag materials from the tea.
 - Weigh the tea on a 0.001 g precision scale, and write the dry weight in the excel file.

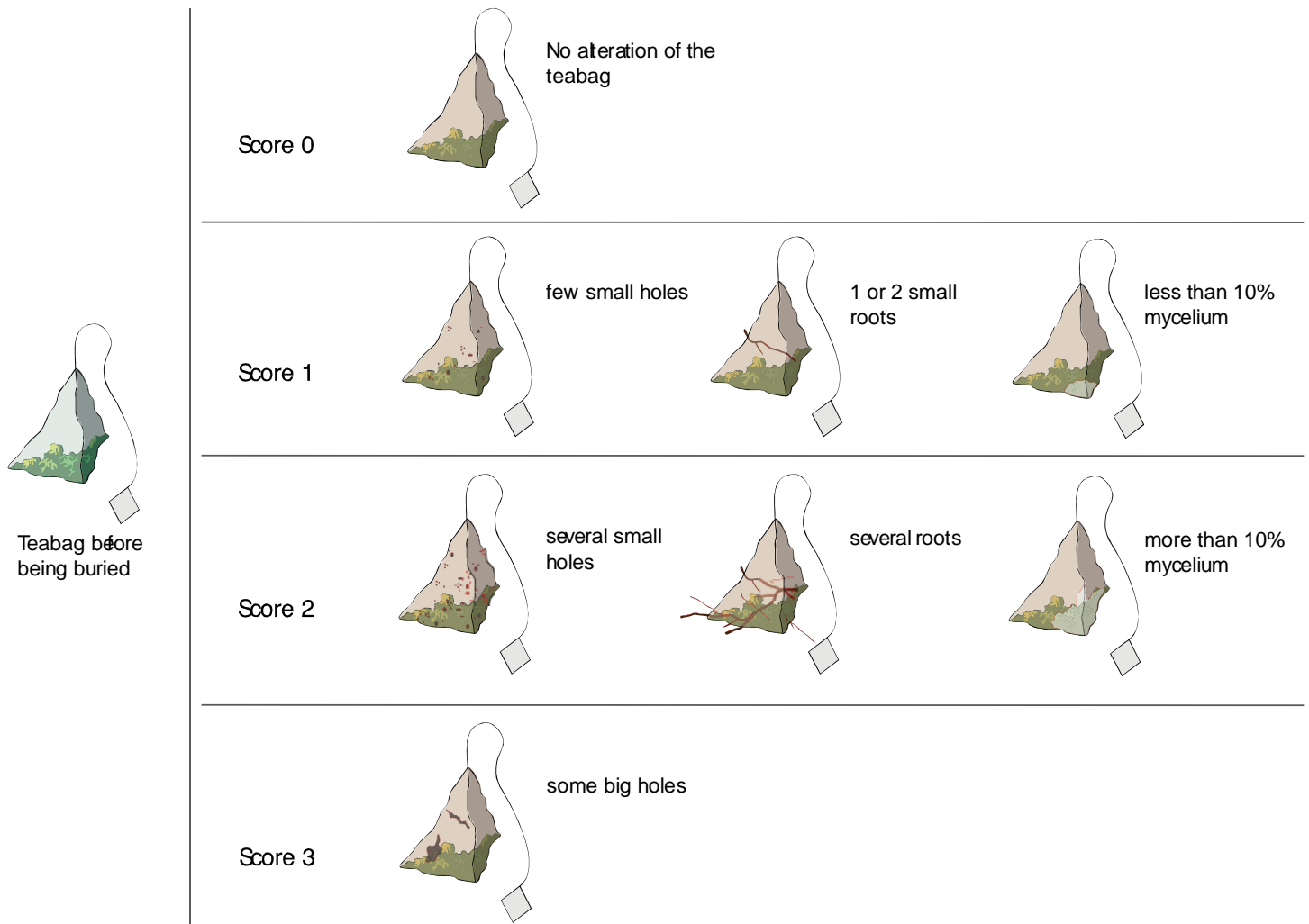


Figure 4: Scoring of the teabags after collection from the field

Data collection

Data should be entered in the provided sheet on the website. Please don't change the column names. If you have questions, just flick us an email. You also find detailed metadata in the dataframe, with units, format of dates and descriptions. All data sheets should be sent by email to Sidonie Loiez and Anne Kempel: sidonie.loiez@ntnu.no ; anne.kempel@slf.ch

The file should be named as follow:

[Site_ID]_teabag.xlm

Screenshot of Metadata in excel sheet:

This is the spreadsheet of the tea-bag addon of the BugNet experiment, bug-net.org	
Lead by Sidonie Loyez	
Teabag_weight	
siteID	Name of the site
plot	Plotnumber, usually from 1-24
tea_type	G = greentea, or R = Rooisbos
replicate	Replicate number, from 1-3 per plot
running_number	A running number (unique number per teabag)
initial_teabag_weight	The initial teabag weight (airdry), that is the entire teabag, with tea, bag and label, in g
date_teabag_in	The date when the tea bags had been burried in the ground; DD-MM-YYYY
date_teabag_out	The date when the tea bags are removed from the ground (ca 3 month later); DD-MM-YYYY
state_teabag	The state of the teabag. 1 = ; 2= ; 3= ; 4= not found
final_teabag_weight_dry	The weight of the tea (dried at 70° for 48 h) without the bag at the end of the experiment, in g
Lab_bags_weight	
Site_ID	Name of site
Label	Label of those bags, L indicates Lab and that they are not burried in the soil
tea_type	G = greentea, or R = Rooisbos
replicate	Replicate number, from 1-10
Purpose	Purpose of those tea bags, "Weigh in lab" is to obtain bag and tea weight
teabag_weight_airdry	Weight of the entire airdried teabag, including tea, bag and lable in g
bagonly_weight_airdry	Weight of the airdried bag only, without tea, string and label, in g
string_weight_airdry	Weight of the airdried string only, in g
label_weight_airdry	Weight of the airdried label only, in g
teaonly_weight_dry	The weight of the tea (dried at 70° for 48 h) without the bag , in g
Transportation_bags_weight	
Site_ID	Name of site
Label	Label of those bags, T indicates that they are used to assess potential effects of Transportation on tea we
tea_type	G = greentea, or R = Rooisbos
replicate	Replicate number, from 1-10
Purpose	Purpose of those tea bags, "Transportation test" is to estimate any potential effects of transportation on
teabag_weight_airdry_before_transportation	Weight of the entire airdried teabag, including tea, bag and lable in g, before the transportation
final_teabag_weight_dry	The weight of the tea (dried at 70° for 48 h) without the bag at the end of the experiment, in g
Temperature_Moisture	
These measurements are optional, but recommended. It depends largely on the an	
siteID	Name of site
plot	Plotnumber, usually from 1-24. The reference logger should be placed in the centre of the plot and indic
Date_start	The date when the logger has been placed in this particular plot. DD-MM-YYYY
Date_end	The date when the logger has been removed from this particular plot. DD-MM-YYYY
Temperature_mean	The mean temperature during this period. Please also store all other information from the loggers, until
Moisture_mean	The mean moisture during this period. Please also store all other information from the loggers, until we