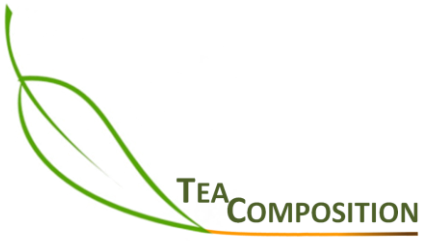
GLOBAL LITTER DECOMPOSITON STUDY



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Litter decomposition represents one of the largest fluxes in the global terrestrial carbon cycle and already diverse large-scale decomposition experiments were focusing on this fundamental soil process. However, these are most often conducted based on site specific litters and methodologies and comparison of similar data across different experiments and sites still poses a major challenge due to the lack of common protocols and standard matrices. The Tea bag method (Keuskamp et al., 2013) is a simple, standardized, cheap and time-efficient method involving 2 types of tea: Rooibos tea characterized by a slow decomposition rate and Green tea characterized by a faster decomposition rate. The advantage is that these teas are commercially available anywhere and the tea bags constitute a pre made "litterbag" reducing any variation related to user differences in preparation. We aim to study the potential litter decomposition by using the standard substrate (tea) for comparison of the long-term decomposition rates across sites. Hence, the focus would be both, on the litter contribution to C-losses and C-storage and their main drivers at the present and predicted climate change worldwide.

This method does not give the actual number for C-losses and decomposition rates, since the tea is not equivalent to the real local litter, but can be related to local rates (e.g. by simultaneous incubation of native litter) and modelling. Therefore, it would be of advantage installing the tea bags together with the local litter bags or using the sites for the installation with already existing data on native litter decomposition.

The method involves measuring a tea bag before and after incubation in the field and using the difference in weight as a measure of the organic material decomposed. This means that it is critical to follow the protocol very closely. For example, **weighing** both before and after is critical and that the installation, **retrieval** and **cleaning** of the bags are critical in order to not lose any tea that will then be mistakenly assessed as being decomposed or not to leave any soil and other "non-tea" remains on the bag after retrieval, which will mistakenly be assessed as "non-decomposed tea". Furthermore, **proposed requirements** (e.g. the start of incubation trial, exposition, soil depth, retrieval times, tea type etc.) **must be kept constant**; i.e. any deviations from the protocol have to be announced and discussed a priori.

Best!

Ika

References

Keuskamp J, Dingemans BJJ, Lehtinen T, Sarneel JM, Hefting MM. 2013. Tea Bag Index: a novel approach to collect uniform decomposition data across ecosystems. Methods in Ecology and Evolution 4: 1070–1075.

TEA BAG PROTOCOL

The Tea Bag method uses 2 types of tea bags from Lipton:

- **Green Tea (EAN no.: 8 722700 055525)** ingredients are: Tea 89%, Flavouring 9.3%, rose Petals 1%
- **Rooibos Tea (EAN no.: 8 722700 188438)** ingredients are: Rooibos sud-africain 93%, arome hibiscus1%

The bags can be ordered from dutchsupermarket.com (use the EAN no. when ordering).

Note: I am in dialog with the UNILEVER company (that produced Lipton tea) in order to develop an efficient and simplified logistic plan for the tea bag supply. I will inform you about the progress and the possibilities before the start of the study.

0. Design of incubation and selection of site/plots/replicates

In order to find out how many tea bags you need, you have to plan and design the incubation in terms of how many sites, plots and replicates are needed. Each incubation involves selection of ONE plot with incubation of 32 tea bags of each type (64 bags in total) in two replicate areas (see Fig.1). This means that for your SITE you have to decide how many incubation plots you want to have either decided by number experimental plots that should be covered or by a demand for replications. For example, if you have a monitoring site where you want to get 4 replicated incubations, you need to install 4 incubation plots with each 32 Green and 32 Rooibos tea bags. Similarly, if you have an experiment with 4 control plots and 4 treatment plots and you want to cover each of these experimental plots with one incubation you need in total 8*(32 Green + 32 Rooibos) teabags.

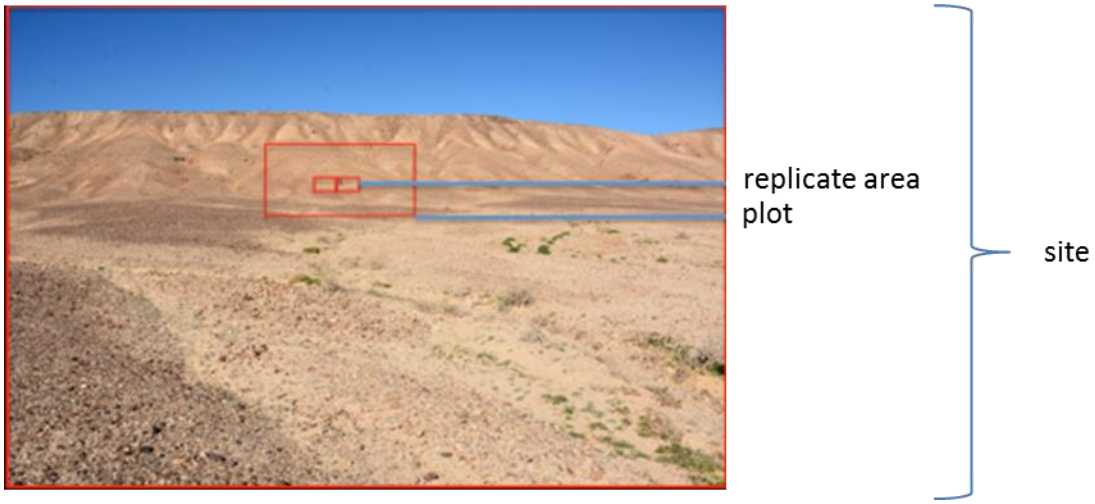

Figure 1: Terms: site/plot/replicate area

1. Preparation of tea bags in the lab

Figure 2: Work steps 1.1. -1.4

1.1. For one plot (see Fig.1 for the clarification of the terms site/plot/replicate in this protocol) dry 32 bags of green and 32 bags of rooibos tea at 70°C for 48 hours.
1.2. Label them on the white side of tag (Fig. 2.2) using a permanent marker with a "unique identifier" (e.g. IL31 = IL stays for Israel, 31 is the 31st sample and you can add G = green tea or R for Rooibos). Additional marking can be used if desirable.
1.3. Before weighing, make sure that the digital bubble on the scale is accurately placed (red circle - Fig.2.3). Weigh them on 4 decimal places (0,000) and note the weight.
1.4. Store the weighed tea bags in a closed plastic bag until the burial (Fig. 2.4). You can collect and store all the tea for one site/plot in a bigger bag (one bag per tea type) signing it with the tea numbers that are inside (e.g. see Figure 3-last photo: "IL 31-44" = tea bag labeling, "1" = site 1, "green tea" = tea type), so in case you have several sites you would have one plastic bag with green and one with rooibos tea per site (i.e. 2 plastic bags per site). In case that there is a risk of loosing tea through the transport you should pack each tea bag individually in a single bag and placing again all these individually packed plastic bags with tea in a bigger bag labeling it as suggested above or threading them on a string (Fig.2.5) Make sure that during the transport, the bags do not get damaged and you do not lose any tea on the way. If this happens, you have to correct the initial weight for the lost amount by reweighing before installation.

2. Installation in the field:

Figure 3a: Work steps 2.2. - 2.6

2.1. Select 2 replicate area in each plot (min. 1m^2 see Figs. 3a.1, Fig.4), where tea bags are going to be installed. If you work with the experimental plots, then the design shall be adapted to fit spatial resources as well as desired research questions. Take a photo (JPEG files; .jpg) of the entire area/site as well as of the plots. The photo should have at least 2000 x 1500 pixel resolution.
2.2. The plot should have a uniform vegetation type of the dominant species and the vegetation type should be similar at both replicate areas (Fig. 3a.2).
2.3. Select a flat spot or if not avoidable a spot with a gentle slope (avoid steep and flat sites along the slope) and describe the topography (see Table 1 as an example).
2.4. Note the coordinates (WGS), elevation above sea level. Selected plots shall have similar exposition; in the Northern Hemisphere the exposition shall be south (south-west/south-east) in the Southern Hemisphere this would be then north (north-west/north-east).
2.5. Describe the vegetation for each area at least at biotope level (see Table 1 as an example). Take a photo of each site.

2.6. Describe soil type (e.g. cambisol, tschernosem), soil depth (form surface to the parent material; see table 1) and parent material (sites should have the same bedrock type). Take a photo of a soil profile (Fig. 3a.3).
2.7. For agricultural sites: Decomposition study can be implemented in the same way at sites with no soil management and with permanent land cover such as permanent grassland. At sites with annual crop rotation and intensive soil management litter bag studies are "difficult" to implement. If you plan to do so, contact me for discussion.

Table 1: Example for site description

Altitude	1920 m asl
Slope	26%
Exposition	200° / S-SW
Position (WGS)	N: 47°36`07,05“ / E: 015°05`37,2“
Topography	Shape of the slope: linear slope, few dolines and outcrop of bedrock. Length of the slope: 300 m. Position: backslope.
Soil	Parent material: limestone; Soil type: Leptosol (IUSS Working Group WRB, 2006). Average soil depth is 19 cm. The litter layer is marginally developed. A 5 cm thick gramineous tomentum layer overlays the soil.
Biotope	Alpine grassland, mountain pine bushes
Tree layer	None
Shrub layer	*Vaccinio myrtilly-Pinetum montanae* / Shrubland dominated by *Pinus mugo*
Herb layer	*Carex firma* grassland / *Festuca pumila-Agrostis alpina grassland* (closed)

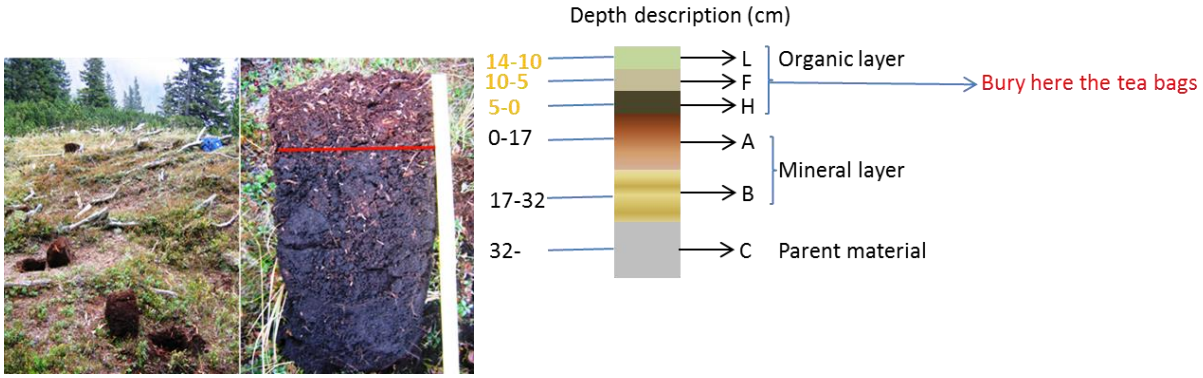

Figure 3b: Work steps 2.7. - 2.9

2.7. Take 3 soil samples (approx. 100g) from the organic soil layer (F-H horizon; ~5-0 cm after removing the litter layer; see Fig. 3b) at each site for the analysis of the main soil properties- **this is only needed if no soil data are available** (see point 5). If there is no organic soil layer, then collect the top soil just underneath the litter layer (H-A layer). The collected soil shall be air dried and 2 mm sieved for the further analyses.
2.8. Start the incubation in June 2016 in the Northern Hemisphere. In tropical region this shall be adjusted to the time of year with the peak of litter fall (December 2016). In the desert area the start shall be October 2016.
2.9. Note the date for the start of the incubation. This is important for the retrieval time, which you shall plan accordingly and remember.
2.9. Install 16 teabags of Green tea and 16 tea bags of Rooibos tea in each area into the organic soil layer (ideally in F layer, or in the lower L/upper H layer, Fig. 3b). If you have a very shallow soil and almost no organic layer, then insert the bags very close to the surface (H-A layer; 0-2 cm) at all sites. With two areas per plot

this means that in total 32 bags of green tea and 32 bags of rooibos tea per plot will be needed (see Fig. 4). Indicate in your soil description (step 2.6) the exact installation depth.

2.10 The 16 bags of each tea shall be installed in 4 "installation lines" with each 4 green and 4 rooibos tea bags. Each line should be 40 cm long allowing 4 teabags to be placed on the line with approximate 10 cm between neighboring bags (Fig. 4). For each "installation line", make 4 times a 5cm deep slot vertical into the soil, then cut horizontal into the soil, lift gently and place the teabag into the soil organic layer (ideally in F layer, or in the lower L/upper H layer, Fig. 3b) so that only tag looks out. Place every additional bag in a distance of approx. 10cm. Place the bags in ascending serial number in a row, so that in case a labeling is missing you can "reconstruct" the missing label number from the numbers of the previous and subsequent bags. For any eventuality, label the beginning and end of 4 bags with metal sticks and number plate so that in case labeling is missing you can assign the bag number when they are placed in ascending serial (see Fig. 4). Metal sticks are also suitable to be detected with metal detector, if the spot is covered with thick litter layer. In case the rope with tag is falling apart from the bag, take a tacker with you to attach them again.

2.11. Mark the replicate areas, so that you can find them easily. Draw a sketch of the teabag set up (Fig.4).

Figure 4: Sketch of the study set up

3. Retrieval

3.1. Plan the retrieval dates in your schedule. Teabags should be retrieved after 3, 12, 24 and 36 months in the following way: At each sampling point, collect 4 tea bags of Green tea and 4 tea bags of Rooibos tea (don't pull on the rope but lift the soil to take out the bags) from each incubation area (one incubation line per sampling point); this results in 8 tea bags from both plot, tea type and sampling time.

3.2. If the bags are damaged, or you found them at the surface, please register/document those observations, which might be relevant for the data processing.

3.3. Place every single tea bag in a separate plastic bag and check the labeling on the tag. If the labeling is badly readable or even missing, reconstruct the number (by checking the previous or following tea bag numbers in the line) and label the tag or bag again.

3.4. Repeat the procedure for retrieval after 12, 23, and 36 months.

4.

Figure 5: Reprocessing of the collected tea

4.1. Clean the tea bags manually from roots, soil etc. (be careful not to lose any tea, and make sure all soil and plant debris are removed to avoid errors in the weighing).
4.2. Dry the bags at 70°C for 48 hours.
4.3. Before weighing make sure that the digital bubble on the scale is accurately placed (Fg.2.3). Weigh each tea bag on 4 decimal places (0,000) and note the weight to the initial weight value.
4.4. Transfer the tea in a glassine paper-bag (7-12 cm), label (county/site/ sample ID/tea type, incubation length, date) and close the glassine bag (Fig. 5). Note if the bag was damaged or found at the surface.
4.5. Weigh the empty tea bag (= bag + string + tag) and register the weight. If you have used taker to fix the rope, remove it before the weighing. In case of bag damage (the loss of string, tag), heavy contamination the weight of the average weight of the fresh bag (0.283 g) could be used for the further calculation. Please state this also when reporting the data.
4.6. In case soil has entered the bags and cannot be easily removed by external cleaning and the measured incubation litter weight is higher than the initial one, then the samples have to be analyzed for the organic matter content and carbon content of the soil by using muffle oven.

5. Data requirement:

In order to be able to interpret and to link the litter mass remaining data to the potential driver of the litter decomposition additional data must be collected. Information to data reporting and data analyses will follow. However, the required data over the incubation period (i.e. June 2016-June 2019) is:

- Annual average air temperature (°C)
- Annual precipitation (mm)
- Annual average temperature amplitude (mean temp. of the warmest month - mean temp of the coldest month)/2)
- Basic soil properties (pH, OC, Ntot); from F-H horizon; ~5-0; only at the beginning of the study

Further desirable data:

- If possible-soil temperature (5 cm depth; recorded daily)
- If possible -soil moisture (5 cm depth; recorded daily)
- If possible, for each sampling time, at least one composite sample per control plot and per treatment shall be made for the following analyses: litter OC, Ntot, P, S, K, Ca, Mg, Mn, tannins, cellulose, hemicellulose, lignin, heavy metals (Cu, Zn, Pb, Cd). If they are more resources, then one sample per replicate shall be measured.
- If possible, soil nutrients (P, S, K, Ca, Mg, Mn) and heavy metals (Cu, Zn, Pb, Cd)- F-H horizon; ~5-0; only at the beginning of the study

I am working on the development of an online data-reporting template, where the required data should be inserted. Anyway, the collected information could also be sent to me vial mail or post:

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ADD-ONS: (*optional*)

6. Litter bags with native litter

6.1. Collect the intact (whole) shed leaves and dry them at 50°C to a constant mass.

6.2. Make the triangular litter bags of polyethylene net (10x10 cm, with a mesh size of 0.25mm)

6.3. Fill each bag with approximately 2 g of single (whole) leave type. Notice the weight and label the bags with an ID.

6.4. Proceed further similar as for the tea bags (points 2, 3, 4, 5).

Figure 6: Litter bag

6.5. If possible for each sampling time, at least one composite sample per control plot and per treatment shall be made for the following analyses: litter OC, Ntot, P, S, K, Ca, Mg, Mn, tannins, cellulose, hemicellulose, lignin, heavy metals (Cu, Zn, Pb, Cd). If they are more resources, then one sample per replicate shall be measured.

6.6. For each litter type the chemical AWEN fractions (1) acid soluble, 2) water soluble, 3) ethanol soluble, and 4) non soluble) shall be determined if they are not listed on the Yasso07 website (http://www.syke.fi/projects/yasso).