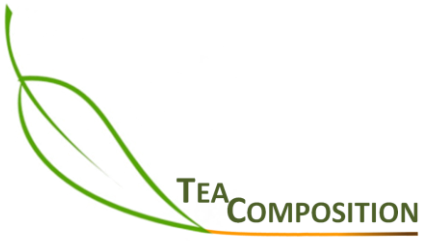
# GLOBAL LITTER DECOMPOSITON STUDY




**Ika DJUKIC**

**Email: ika.djukic@umweltbundesamt.at**

**2016/08/02**

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 and the tea bags constitute a pre made "litterbag" reducing any variation related to user differences in preparation. With the TeaComposition initiative, we aim to study the long-term litter decomposition and hence the **long-term C dynamics** (both the litter C-losses and C storage) and its **key drivers at the present and predicted climate** scenarios worldwide. The TeaComposition method is a modified method published by Keuskamp at al. 2013. The modifications in the TeaComposition method are:

- Incubation length: We aim to run the tea incubation over the period of 3 years with several sampling points in order to get data on the medium-to-long term litter decomposition rates. By running the experiment over years instead of months we overcome the problem with seasonality and timing, which can be an issue with short term incubations, and we believe that we get more robust values for the given site/ecosystem.
- Incubation depth: tea is incubated at specific soil layer rather than at a certain soil depth, since the "required depth" can vary drastically from site to site and from ecosystem to ecosystem.

Specified start of the study: we aim to start the study at the same time of the year (start in northern hemisphere and southern hemisphere will be adjusted accordingly).

Tea supply: UNILEVER, the company that produces the Lipton tea, is sponsoring the "TeaComposition" initiative, so that all sites will receive the same batch of tea and hence assuring the primary criteria of having the same substrate quality for all sites.

Network approach: The "TeaComposition" initiative is an initiative that seeks to use existing infrastructures of the global networks and their data bases relevant for understanding and addressing decomposition process and thus shall be seen as a cost-efficient "Add-On".

Funding and resources: The TeaComposition initiative does not have funding to pay for the effort for incubation, retrieval, cleaning and weighing and eventually chemical analyses at the sites. The initiative is an "offer for global collaboration, coordination and comparison" which several hundred sites have already accepted. There are a number of significant benefits provided from the initiative (outlined below) and we trust that the request for manpower is rather limited for each and well worth the effort to obtain this global link and inclusion and to obtain results for the site that most sites would need anyway. But, it is has to be remembered that there is a request for some resources and especially commitment from the sites.

Benefits:

1) This method provides a common metric for studying decomposition and C dynamics and storage. The common metric will provide a strong tool and results for inter-site comparison within the network as well as with other global networks.

2) By obtaining harmonized data on one of the basic soil processes we will be able to draw general conclusions on the impact of climate and other drivers on litter decomposition and thereby on the greenhouse gas emissions and terrestrial feedback as well as soil carbon storage in different ecosystems worldwide.

3) Common dataset for the network related to decomposition and C turnover which can be used for syntheses and analyses and for reference to other studied factors.

4) The results should potentially provide data for high-impact joint publications and for model application and validation.

Workload and resources:

The site has to provide manpower and resources for:

- Installation of the teabags and retrieval (collection) after incubation
- Cleaning and weighing of the tea bags after incubation
- providing standard information about the site (standard and generally available for most sites)
- Optional: parallel running of a litterbag study with local litter
- Optional: Chemical analyses of tea material and soil (no resources can be provided but a common project may be applied for to cover this)

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 important 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 UNILEVER is sponsoring the TeaComposition initiative. After assignment in the file available under circulated link, the tea will be dispatch to your address.

### 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 (treatments) are needed. Each incubation involves the selection of ONE site with incubation of 16 tea bags of each type (32 bags in total) in two replicate areas (see Fig.1). For example, if you have one site you need to install 2 replicate areas with each 2 Green and 2 Rooibos tea bags per replicate area and sampling point i.e. (1*(2 Green+ 2 Rooibos ) *2 replicate area *4 sampling points); in total this would be 16 tea bags of green tea and 16 tea bags of Rooibos tea. 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 treatments*(2 Green + 2 Rooibos) *2 replicate area *4 sampling points) resulting in total of 128 teabags of green tea and 128 of Rooibos tea. For the dessert area, the incubation length could be extended for the additional 4 years, because of expecting slow decomposition rate: i.e: (1*(2 green+2 Rooibos)* 2 replicate area *8 sampling points).

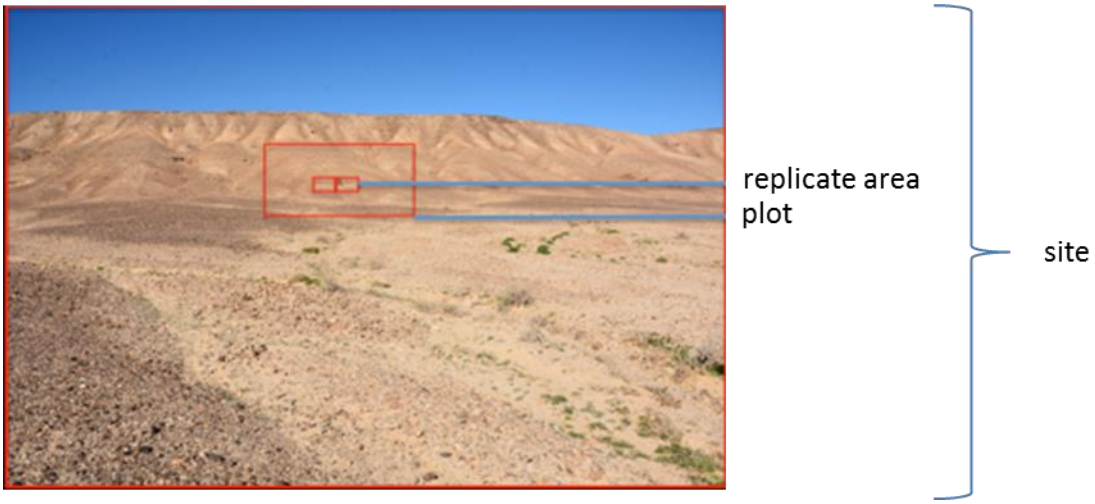



*Figure 1: Terms: site/plot/replicate area*

## 1. Preparation of tea bags in the lab

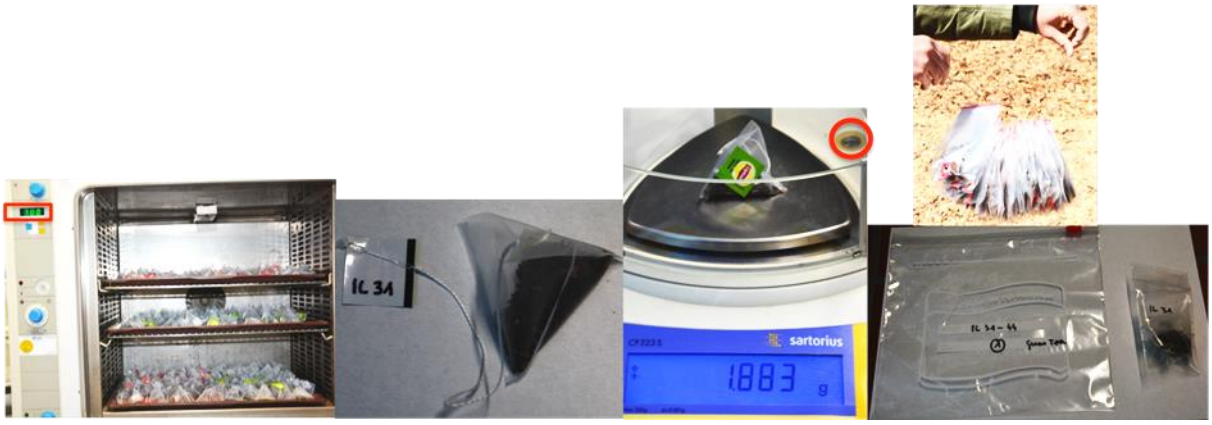



*Figure 2: Work steps 1.1. -1.4*

1.1. For one site (see Fig.1 for the clarification of the terms site/plot/replicate in this protocol) dry 16 tea bags of green and 16 tea bags of rooibos tea at 70°C for 48 hours. For desert area, you will need 32 tea bags of green and 32 tea bags of rooibos tea.

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 31$^{st}$ 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 preferably on 4 decimal places (0,000) and note the weight.

1.4. Store the weighed tea bags in a zip-lock bag until the burial (Fig. 2.4). You can arrange and store all the tea for one site in a bigger bag (one bag per tea type) labeled 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); for each of your four site you would have one plastic bag with green and one with rooibos tea (i.e. 2 plastic bags per site). In case that there is a risk of losing 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. Plastic boxes are suitable for a safe transport. If this happens, you have to correct the initial weight for the lost amount (remaining in the plastic bag) by reweighing its content upon being back in the lab.

## 2. Installation in the field:

2.1. Select 2 homogeneous replicate areas (min. 1m$^2$) at each site 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 resolutions.

2.2. The site should have a uniform vegetation type of the dominant species and the vegetation type should be similar at all replicate areas.

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 replicate areas 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. 3). Please check also this link for further help: http://www.fao.org/3/a-i3794e.pdf.

*Figure 3: An example for the soil profile*

2.7. For agricultural sites:

- At sites with no soil management and with permanent vegetation cover such as permanent grassland the decomposition study can be implemented in the same way. If the sites are managed, than I would suggest having at least two plots:
  1) One plot with no mowing, no grazing and no fertilizer application
  2) One plot with 1-2x mowing (manual), no grazing and no fertilizer application
  3) Additional combinations are possible and welcome but in order to harmonize the data each site shall provide at least one site as stated in point 1 or 2.

- At sites with annual crop rotation and intensive soil management the study shall be adapted and stick to following criteria:
  1) Install the tea bags in the field when the crop is on the field (not into the bare land)
  2) Consult with the farmers about the sequence of the crops, their fertilizer requirement as well as soil tillage and choose the fields with the most similar crops sequences and treatments.
  3) Incubate the tea for 3 months and repeat the installation during the next 3 years

| | |
|---|---|
| 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 myrtill-Pinetum montanae* / Shrubland dominated by *Pinus mugo* |
| Herb layer | *Carex firma* grassland / *Festuca pumila-Agrostis alpina grassland* (closed) |

*Table 1: Example for site description*

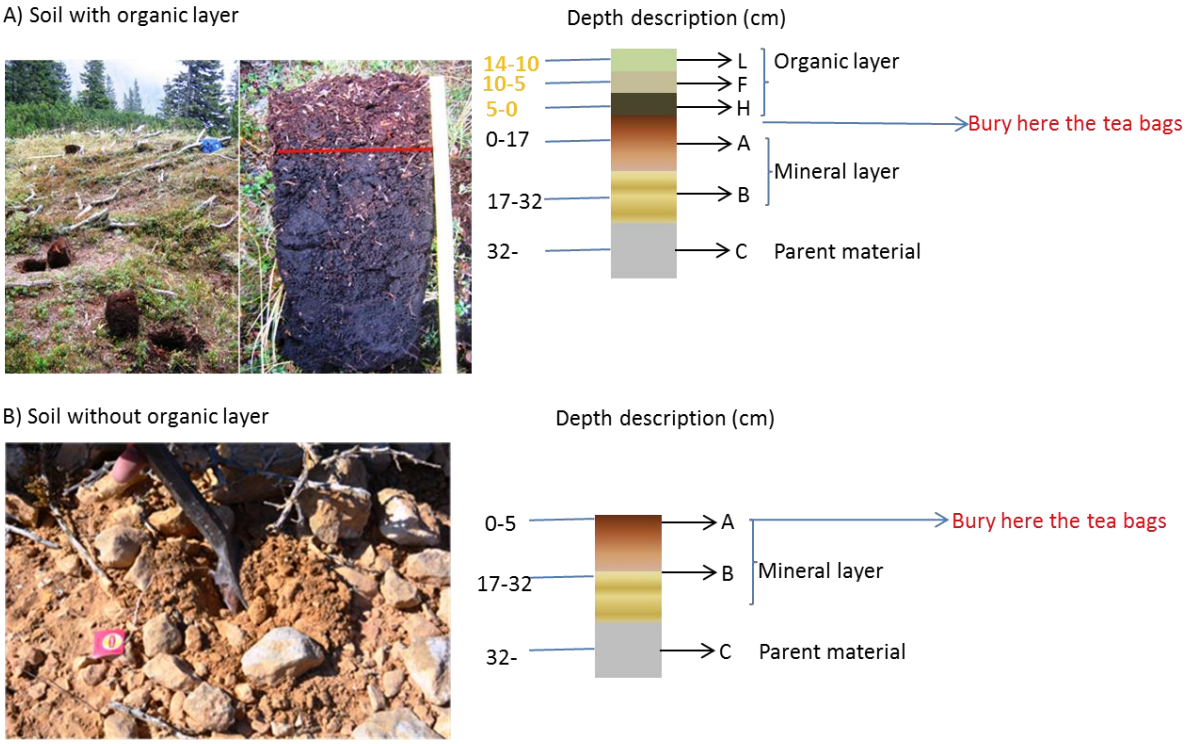



*Figure 3b: Work steps 2.7. - 2.9 – Positioning of tea bags in the soil. Soil layers: a) organic layer (L =leaf litter layer, F =fermentation layer-where organic matter starts to decompose, H = humus; b) mineral layer (A = topsoil- mixture of organic matter and mineral matter, B =subsoil; c) parent material*

2.8. Take 3 soil samples (approx. 100g) from the Ah mineral soil layer (~0-5 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). The collected soil shall be air dried and 2 mm sieved for the further analyses. The soil collection can be performed at any sampling point during the 3 years of incubation.

2.9. Start the incubation in June 2016 in the Northern Hemisphere and in December 2016 in southern hemisphere, respectively. In the desert area the start shall be October 2016.

2.10. Note the date for the start of the incubation. This is important for the retrieval time, which you shall plan accordingly and remember.

2.11. Install 8 teabags of Green tea and 8 tea bags of Rooibos tea in each replicate area into the Ah mineral soil layer (0-5 cm, Fig. 3b). With two replicate areas per site this means that in total 16 bags of green tea and 16 bags of rooibos tea per site will be needed (see Fig. 4). Indicate in your soil description (step 2.6) the exact installation depth. In desert area install 32 teabags of Green tea and 32 tea bags of Rooibos tea in each replicate area; i.e. in total this would be 64 of green tea and 64 of Rooibos tea.

2.12. At each replicate are install 2 green and 2 rooibos tea bags in 4 “installation lines”. 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 Ah mineral soil layer

(~0-5cm, Fig. 3b) - the tag shall be visible on the surface. 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. Optionally, 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 string with tag is falling apart from the bag, take a tacker with you to attach them again.

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

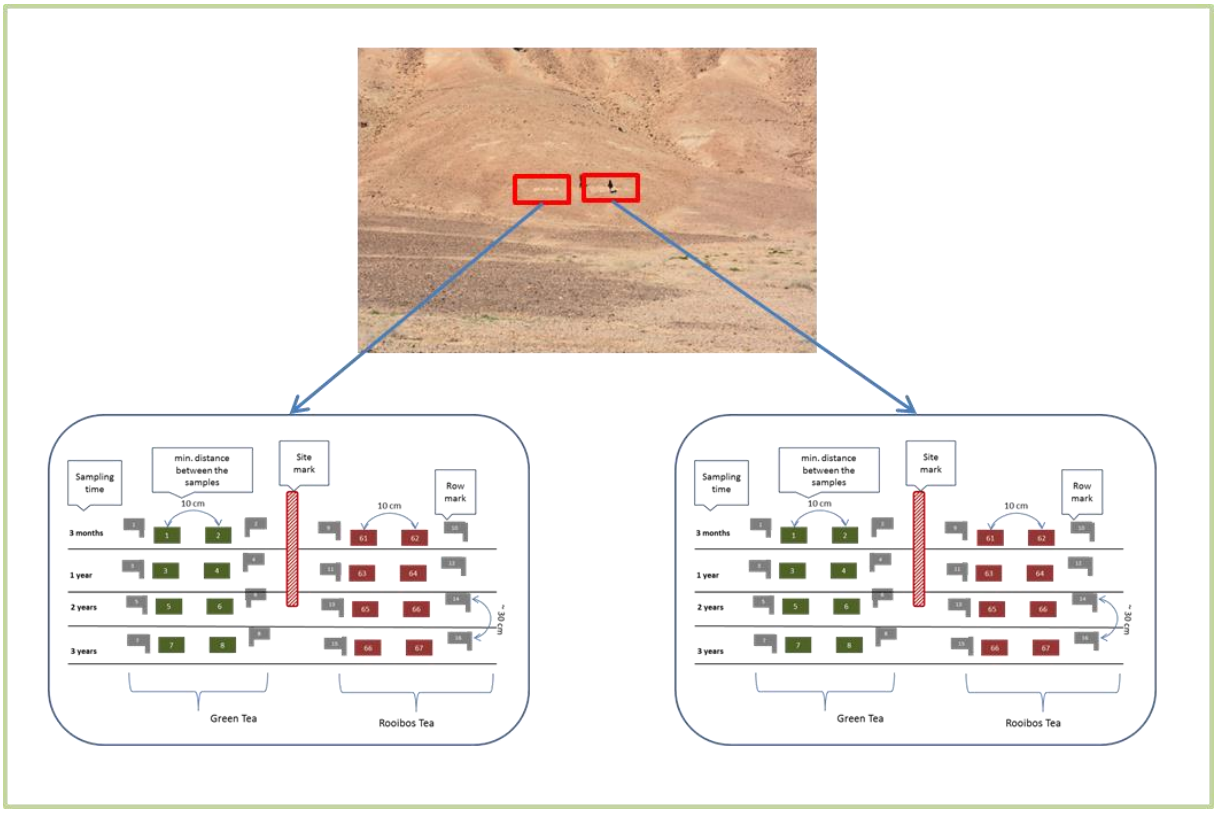



*Figure 4: Sketch of the study set up. Note adjustment for the desert area.*

## 3. Retrieval of tea bags

3.1. Plan the retrieval dates in your schedule in accordance to the data of installation. Teabags should be retrieved after 3, 12, 24 and 36 months (and after 48, 60, 72, 84 months in the desert area) in the following way: At each sampling point, collect 2 tea bags of Green tea and 2 tea bags of Rooibos tea (don't pull on the rope but lift the soil to take out the bags) from each replicate area (one incubation line per sampling point); this results in 4 tea bags of each tea type per site 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 (and after 48, 60, 72, 84 months in the desert area).

## 4. Reprocessing of the tea in the lab:

*Figure 5: Work steps 4.1. – 4.6 – Reprocessing of the collected tea.*

4.1. Start reprocessing of the tea bag in the lab as soon as possible (preferably within 1 week after collection of the sample)

4.2. 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).

4.3. Dry the bags at 70°C for 48 hours. If necessary, remove remaining soil.

4.4. Before weighing make sure that the digital bubble on the scale is accurately placed (Fg.2.3). Label the glassine paper bag (county/site/ sample ID/tea type, incubation length, date) and tare weight them. Open the tea bag and transfer the tea into the glassine paper bag. Weigh the glassine paper bag with tea preferably on 4 decimal places (0,000) and note the weight. Close the glassine bag with a stripe of the sellotape.

4.5. Note if the bag was damaged or found at the surface.

4.6. Use default value (0.248 g) for the empty tea bag

4.7. 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 (at 500°C overnight (16h)).
Mineral content [%] = (RW/ODW) x 100; where: RW = Residue weight after ignition
ODW = Oven-dry soil weight. Organic matter percent [%] =100-Mineral content [%]

## 5. Required additional data:

In order to be able to interpret and to link decomposition data to the potential driver of the litter decomposition additional data are needed. The minimum required data over the incubation period (i.e. June 2016-June 2019) are:

- 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)

Note: If climatic data are not available at site, please provide the most reasonable meteorological data from the area.

*Further desirable data:*

- If possible-soil temperature (5 cm depth; recorded daily)
- If possible -soil moisture (5 cm depth; recorded daily)
- If possible, basic soil properties (pH, OC, Ntot ); soil nutrients (P, S, K, Ca, Mg, Mn) and heavy metals (Cu, Zn, Pb, Cd); Ah mineral layer; ~0-5 cm; only ones during the 3 year of incubation.
- Optional: 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.

Information to data reporting and data analyses will follow. 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 via email or by mail:

Ika Djukic
Environment Agency Austria
Brigittenauer Lände 50-54 (3th floor)
1203 Vienna, Austria
E-mail: ika.djukic@umweltbundesamt.at

## 6. Outputs, benefits, data and IPR

The data will be collected in a common database with conditional access for all to data from the network. This database will allow syntheses and assessment of the tea decomposition across the network (Objective 1) and including the evaluation of the influence of key drivers of change (climate, soil, management, tree species, plant diversity etc.; Objective 1 and Objective 3) on key processes and potentially the relationship of this tea dynamic to local litter quality (Objective 2). In addition, the database will be aligned with similar databases for other corresponding ecosystem networks allowing for global analyses and model applications (Objective 4).

The data will be available to all partners in the network who contributed and can be conditionally used meaning that open processes will be applied where everyone will be notified and asked for the use of their data and with a chance to interfere.
It is intended that one high level syntheses paper will be produced based on this activity and all sites who contributed will be offered co-authorship for this. For further papers, we will adopt the same data policies as used in other global networks (ILTER, NutNet, Drought-Net etc.) and follow the Vancouver guidelines. This means that site data can be used by others without automatically leading to co-authorship rights accepting that co-authorship requires academic and scientific input that is not fulfilled by contributing data alone. Further co-authorship rights therefore require more substantial inputs than just the data.
In summary, the outputs are:

- Common database for the network with access for all data contributors
- Aligned database with other corresponding global databases
- One High-level publication including all participating partners. Deadline for the publishing the outcomes of short term litter data (3 months) should be within 1 year after collection of data.
- Future possibilities for local, regional and large-scale analyses of short (3 months) and long term (up to 3 yrs) litter and carbon dynamics related to key drivers of change
- Future possibilities for model collaboration based on tea and local litter dynamics.

### ADD-ONS: (*optional*)

The TeaCompositon method does not give the actual magnitude 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 modeling. Therefore, it would be of advantage installing the tea bags together with the local litterbags or using the sites for the installation with already existing data on native litter decomposition.

## 7. Litter bags with native litter

7.1. Collect the intact (whole) shed leaves from two dominant species (with different litter quality) and dry them at 70°C to a constant mass.

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

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

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


*Figure 6: Litter bag*

7.5. If possible at 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.

7.6. If possible 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).

7.7. The methods for the suggested analyses will be notified and possibilities about running the analysis at one lab will be discussed.