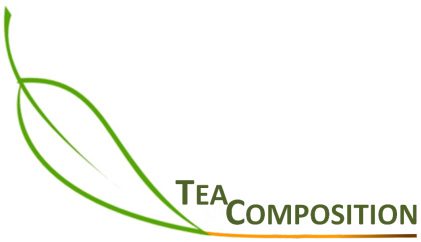
GLOBAL LITTER DECOMPOSITON STUDY



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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 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**. 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 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 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 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 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. Due to the common metric it 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 green house 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:

- Drying, weighing and 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.

TEACOMPOSITION 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 by providing the tea bags out of the same batch. Each GLORIA site (target region) usually consists of four summits. For each summit you need 32 tea bags (16 green and 16 rooibos), thus a total of 128 bags (64 for each tea type) are required. Sign up your sites in the file under the link below: and provide your shipping address

https://docs.google.com/spreadsheets/d/1e74dYFqBlO7siWjLNgqwj3cnTE8mzGucLQsKfgYbOtM/edit?usp=sharing

The tea will be dispatched to your address.

1. Preparation of tea bags in the lab

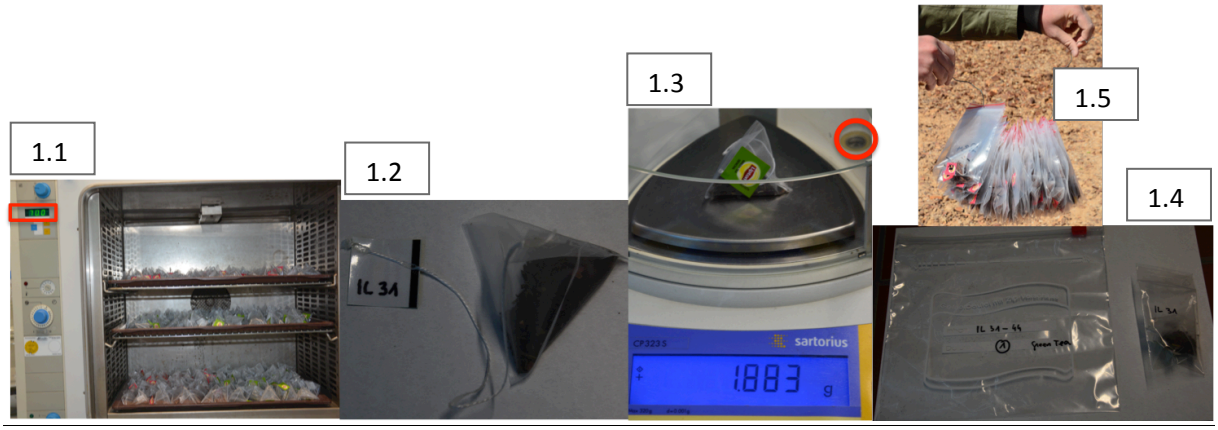

Figure 1: Work steps 1.1. -1.4

1.1. For your four summits dry 64 bags of green and 64 bags of rooibos tea at 70°C for 48 hours (Fig. 1.1). If there is no lab oven available, drying in a conventional oven is conceivable.

1.2. Label them on the white side of the tag (Fig. 1.2) using a permanent marker with a “unique identifier" (e.g. AT_HSW_WEK_01_G AT=country code, HSW = target region code, WEK=summit code, sample number; tea type). 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.1.3). Weigh them preferably on 4 decimal places (0,000) and note the weight.

1.4. Store the weighed tea bags in a closed zip-lock bag until the burial (Fig. 1.4). You can arrange and store all the tea for one summit and plot in a bigger bag (one bag per tea type) labeled with the tea numbers that are inside; for each of your four summits you would have one plastic bag with green and one with rooibos tea (i.e. 2 plastic bags per summit). 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. 1.5). 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 damage still 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

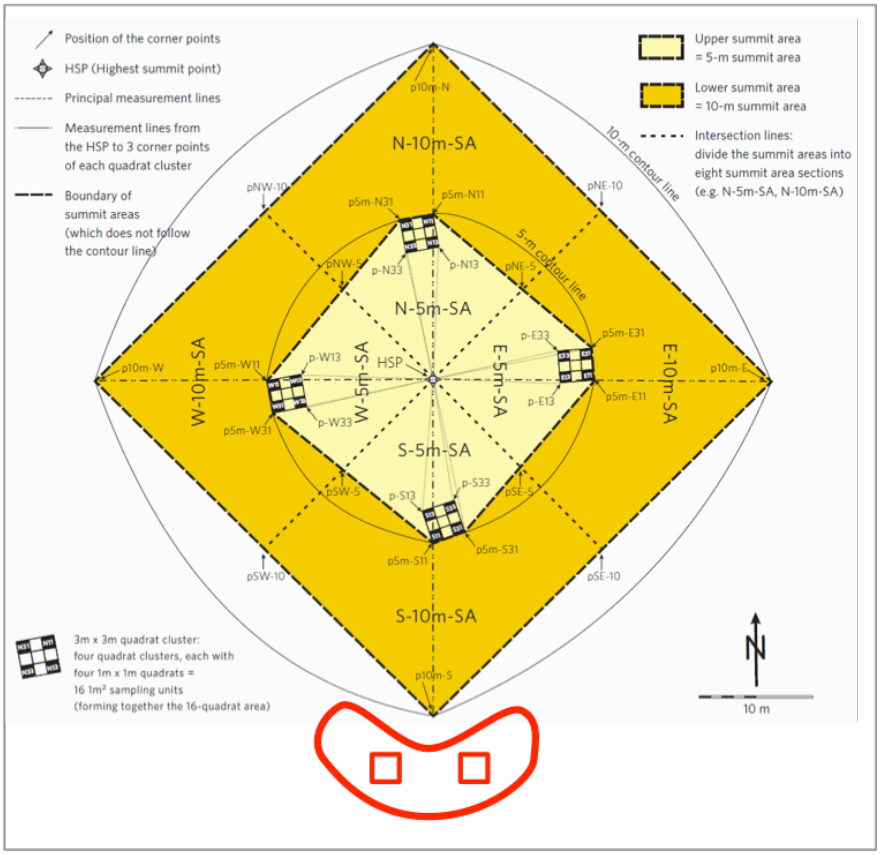

Figure 2: Position of the tea bag plots outside of the GLORIA summit

2.1. At each summit select 2 plots (~1m^2) outside the summit area sections (i.e., at least 2 m below the lower boundary of the 10m summit area section), where tea bags are going to be installed (Fig. 2). Selected plots shall have the same exposure: in the Northern Hemisphere the exposure shall be south (south-west to south-east) in the Southern Hemisphere this would be then north (north-west to north-east).

2.2. The plots should have a uniform vegetation type.

2.3. Select a flat spot on a gentle slope and describe the topography (see Table 1 as an example). The inclination at all 4 summits should be approximately similar (~20° +/- 5°).

2.4. Note the coordinates (World Geodetic System, WGS) and elevation above sea level in meters.

2.5. Describe the vegetation for each site at least at biotope level (see Table 1 as an example). Take a photo of each of each plot per summit and an overview photo that includes both plots and the surrounding area.

Figure 3: An example for a soil profile

2.6. Describe soil depth 50x50cm from surface (if possible) to the parent material (see Table 1). The parent material should have the same bedrock type across all summit sites. Take a photo of a soil profile (Fig. 3) and close the soil profile afterwards. Make the soil description before you start with the incubation of tea bags, so that you are aware of the correct tea bag position in the soil profile (compare Figure 4).

Table 1: Example for the site description

Country Code - Target region Code - Summit Code	AT-HSW-ZIK
Altitude	1920 m asl
Slope	24 degree
Exposure (aspect)	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.
Soil	Parent material: limestone; Soil depth is 19 cm. Litter layer: existing, 5 cm thick Installation layer and depth: Ah mineral horizon, 0-4 cm below litter layer
Biotope	Alpine grassland, mountain pine bushes
Tree layer	None
Shrub layer	Vaccinio myrtillo-Pinetum montanae / Shrubland dominated by *Pinus mugo*
Herb layer	*Carex firma* grassland / *Festuca pumila-Agrostis alpina grassland* (closed)

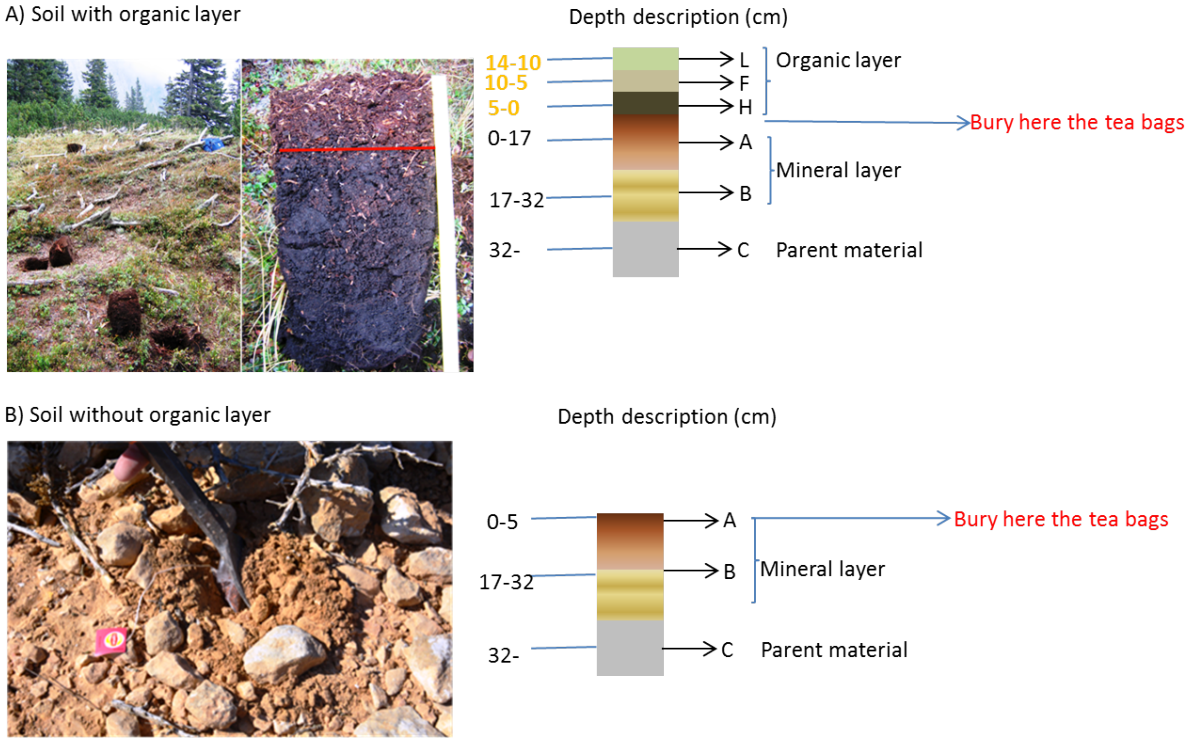

Figure 4: Work steps 2.7. - 2.9 - Positioning of tea bags in the Ah mineral layer (0-5 cm) on the example for the soil with organic layer (A) and without organic layer (B). Soil layers: a) organic layer (L =leaf litter layer, F =fermentation layer-where organic matter starts to decompose, H = humus); b) mineral layer (A = topsoil-mixture of organic matter and mineral matter, B =subsoil); c) parent material.

2.7. Take (randomly) 3 soil samples (each approx. 100g dry weight; approx. two handfuls) from the Ah mineral soil horizon (~2-5) cm after removing the litter layer; see Fig. 4) at each summit for the analysis of the main soil properties 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. The alternatives for dispatching the soil samples will be discussed later on.

2.8. Start the incubation in June to July 2016 in the Northern Hemisphere and in December to January 2016 in southern hemisphere, respectively.

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

2.10. Install 8 tea bags of Green tea and 8 tea bags of Rooibos tea in each plot between the H and the A horizon, i.e. Ah mineral horizon (in ~2-5 cm after removing the litter layers; Fig. 4). The teabag plots must be **at least 2m below the p10m-S** (or **N** in southern hemisphere). With 2 plots per summit this means that in total 16 bags of green tea and 16 bags of rooibos tea will be needed per summit (see Fig. 5). Indicate in your soil description (step 2.6) the exact installation depth.
Note: Given that the summits at most GLORIA sites (target regions) are distributed from the treeline ecotone to the upper zone where vascular plants still occur, soils with and without organic layer will be a common case.

2.11. The 8 bags of each tea (per plot) shall be installed in 4 “installation lines” with each 2 green and 2 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. 5). For each “installation line”, make 4 times a 5 cm deep slot vertical into the soil, then cut horizontal into the soil, lift gently and place the teabag into the position between the H and A mineral soil horizon (see above, Fig. 4); the tag shall be on the surface. To close the slot, press firmly on the surface of the cut. Place every subsequent 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 (as shown in Fig. 5). Additionally, you may mark the beginning and end of 4 bags with small wooden 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). In case the string with tag is falling apart from the bag, take a stapler with you to attach them again.

2.12. Mark the replicate areas, so that you can find them easily. Take a photo (JPEG files; .jpg) of each tea bag plot and of the area around the plots. The photo should have at least 2000 x 1500 pixel resolution. Draw a sketch of the teabag set up (Fig.5).

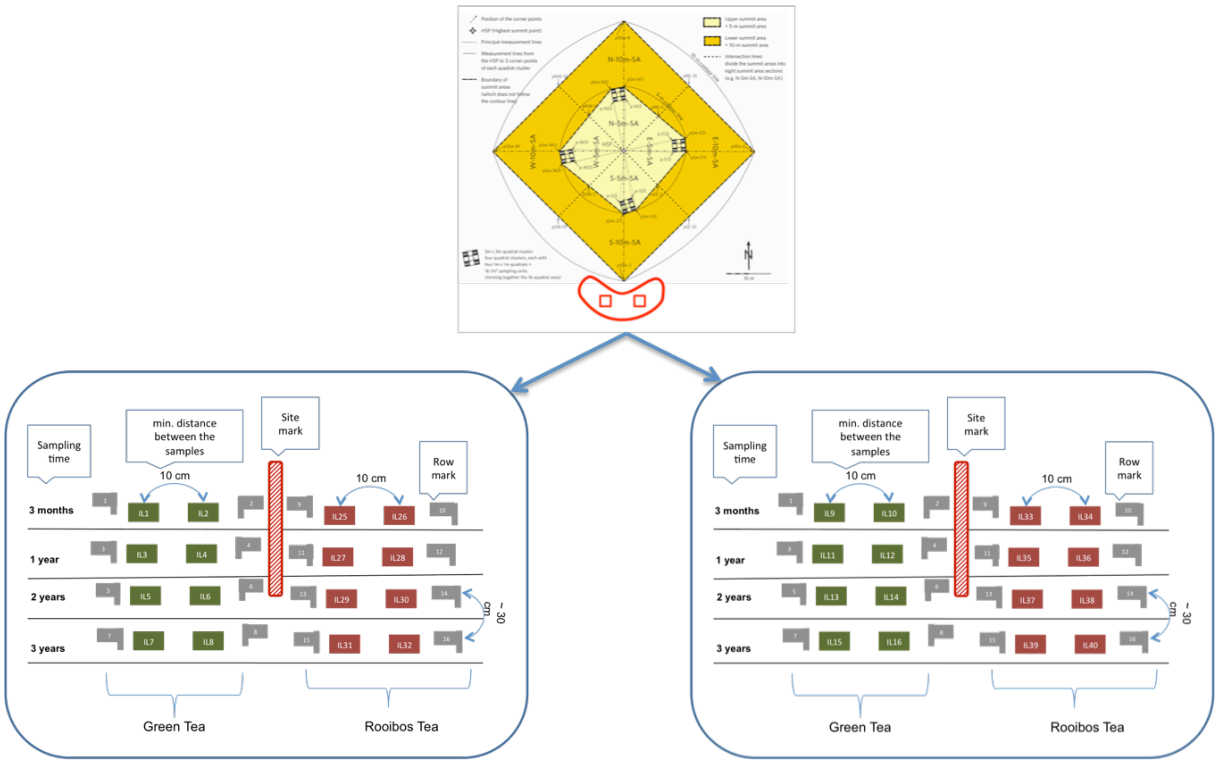

Figure 5: Study design for a GLORIA summit with two tea bag plots, each with 8 Green tea and 8 Rooibos tea bags. Plots must be positioned at least 2m outside of the 10-m point in the southern direction (or the northern on the Southern Hemisphere).

3. Retrieval of tea bags

3.1. Plan the retrieval dates in your schedule in accordance to the date of installation. Teabags should be retrieved after 3, 12, 24 and 36 months 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 plot (one incubation line per sampling point); this results in 4 tea bags per tea type and sampling time and summit.

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, 24, and 36 months.

4. Reprocessing of the tea in the lab

Figure 6: Work steps 4.1-4.6

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). Note if the tea bag was damaged.

4.2. Dry the bags at 70°C for 48 hours and weigh them immediately along the following procedure:
Before weighing, make sure that the digital bubble on the scale is accurately placed (see Fig. 1.3). Label the glassine paper bag (county/site/ sample ID/tea type, incubation length, date) and tare weight them (6.1). Open the cleaned tea bag (6.2, 6.3) and transfer the tea into the glassine paper bag (6.4). 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.

4.3. Determine the weight of the empty "incubated" tea bag by weighing the empty tea bag (bag + string) and note the weight. If you have used stapler to re-fix the string, remove it before the weighing.

4.4. At the end of the entire incubation period, the remaining tea stored in the glassine paper bag shall be dispatched to the laboratory (will be decided) for the further analyses. Also the soil samples taken under point 2.7 shall be sent to the laboratory for analysis.

5. Required additional data

In order to be able to interpret and to link decomposition rates 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) 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.

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 (10 cm depth; recorded daily)
- If possible, soil moisture (10 cm depth; recorded daily)
- If possible, basic soil properties (pH, OC, Ntot, P, S, K, Ca, Mg, Mn) and heavy metals (Cu, Zn, Pb, Cd)- Ah mineral horizon; ~0-5 cm; only once during the 3 year of incubation (compare point 2.7).
- Optional: For each sampling time, at least one composite sample of tea per tea type and per control site/plot and one composite tea sample per tea type and per treatment site/plot. These samples

should be analysed for litter OC, Ntot, P, S, K, Ca, Mg, Mn, tannins, cellulose, hemicellulose, lignin, heavy metals (Cu, Zn, Pb, Cd). If resources are available, 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:

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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 objectives 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 (objectives 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 within GLORIA network as well as within other global networks 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 based on short (3 months) and long term (up to 3 yrs) data. Deadline for 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

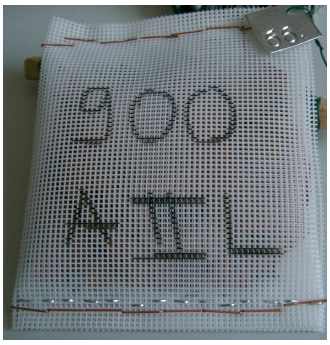

Figure 7: Litter bag

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

7.2. Make the 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.

7.5. 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 there are more resources, then one sample per replicate shall be measured.

7.6. 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).

7.7. The methods for the suggested analyses will be circulated and possibilities about running the analysis at a particular lab will be discussed.