GLOBAL LITTER DECOMPOSITON STUDY

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LITTER DECOMPOSITION STUDY FOR TREEDIVNET

This protocol describes a global initiative for the study of decomposition and carbon dynamics and C turn over across the TREDIVNET network (to be run in parallel with several other global networks). The overarching goal is to draw the conclusions on the general site conditions as well as the effect of tree biodiversity on the litter decomposition patterns across sites in different biomes and how the decomposition patterns and C turn over are affected by litter quality, climate, soil and other site conditions.

OBJECTIVES

OBJECTIVE 1 **To determine the litter decomposition dynamic and contribution of the litter fall to the annual soil C turnover and sequestration.**
(High priority)

More specifically:

1. Effect of stand conditions, tree species and tree diversity on the relative carbon turn over rates
2. Effect of forest management practices on the relative rate of carbon turn over and decomposition
3. Relative control of key drivers like water, light and temperature on decomposition rates and the role of management of these

This will be achieved by installing tea bags at the sites/plots with the tress of the same species or the same mixture along the diversity gradient.

OBJECTIVE 2 **To link the potential to actual litter decomposition at the site**

(Intermediate priority)

More specifically:

1. Relating tea bag litter quality in terms of chemistry and content of lignin, cellulose and other elements to local litter decomposition rates and dynamic
2. Providing a response curve for litter quality and decomposition rate for potential prediction of local litter decomposition based on tea decomposition

This can be achieved by simultaneous installation and thereby parallel incubation of litter bags with the native litter at all or some of the sites/plots along the diversity gradient. This will require extra ressources from the site to collect the native litter, prepare litterbags and incubate and retrieve these. The decomposition numbers from the native litter will however also be very relevant for the sites to provide information on site specific decomposition rates.

OBJECTIVE 3 **To estimate the effects of fertilization and/or irrigation (drought) (or other treatments) on the decomposition and thus soil C turn over and sequestration**

(Low priority) This will be achieved by installation of tea bags at the manipulated sites/plots in parallel with the tea bag installations along the diversity gradient. For proper

treatment comparison installations must be at sites/plots with similar tree species or mixtures as the sites/plots to be compared with.

OBJECTIVE 4 **To provide a framework for comparison of decomposition rates and carbon turn over dynamics across the TreeBioDiv network and with other global networks of ecosystem observations and experiments.**

(high priority) This is achieved by installation according to the common protocol, at corresponding time intervals and by sharing data within a common database allowing multiple data analyses and syntheses to be carried out.

General remarks:

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 lack of common protocols and standard matrices. 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 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 the certain soil depth, since the depth can vary from site to site and from ecosystem to ecosystem drastically.

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 produce the Lipton tea, is sponsoring the "TeaComposition" initiative, so that all site 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. If a site does not see the benefit and do not want to provide the limited resources needed, there is no reason to join.

Benefits

1) This method provides a common metric for studying decomposition and C dynamics and storage. Due tot he 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 conclusion 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:

- Shipping costs for the tea from Austria (Unilever sponsors the tea)
- Installation of the teabags and retrieval (collection) after incubation
- Cleaning and weighing of the tea bags after incubation
- providing a number of standard informations 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 fort o 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.

FIELD 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 attached list, the tea will be dispatch to your address.

1. Preparation of tea bags in the lab

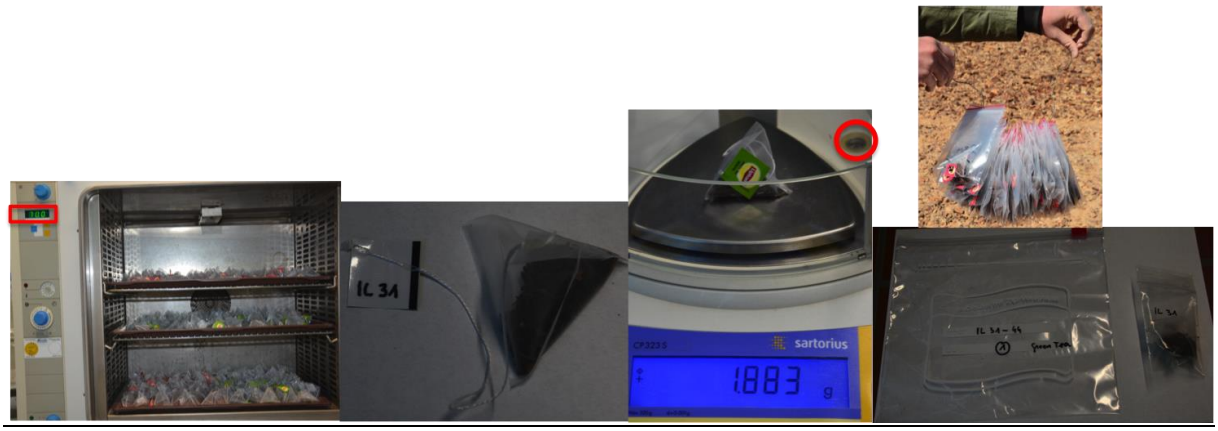

Figure 1: Work steps 1.1. -1.4

1.1. For one plot dry 16 bags of green and 16 bags of rooibos tea at 70°C for 48 hours.

1.2. Label them on the white side of tag (Fig. 1.2) 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.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 plastic bag until the burial (Fig. 1.4). 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.1.5) Make sure that during the transport, the bags do not get damaged (e.g. by transporting them in a plastic box) 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:

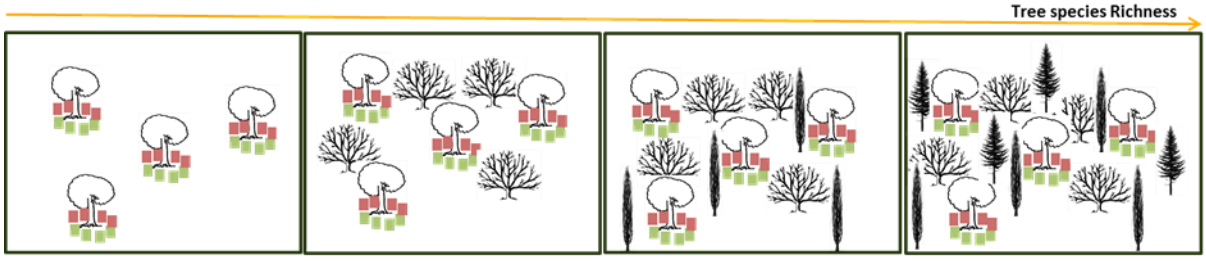

Figure 2: Tree diversity gradient

2.1. Along an increasing tree diversity gradient (i.e. low-intermediate-high diversity plots for three focal species), select 4 trees in each plot (Figs. 2, Fig.4), where tea bags are going to be installed. Selected plots shall have the same 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) 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. 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).

2.3. 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).

2.4. Describe the vegetation for each area at least at biotope level (see Table 1 as an example). Particularly important that the description focuses on the characteristics and differences between the plots/sites that is expected to drive changes/differences in decomposition. Take a photo of each site.

2.5. 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).

Figure 3: Soil profile

Table 1: Example for site/plot 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.
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)

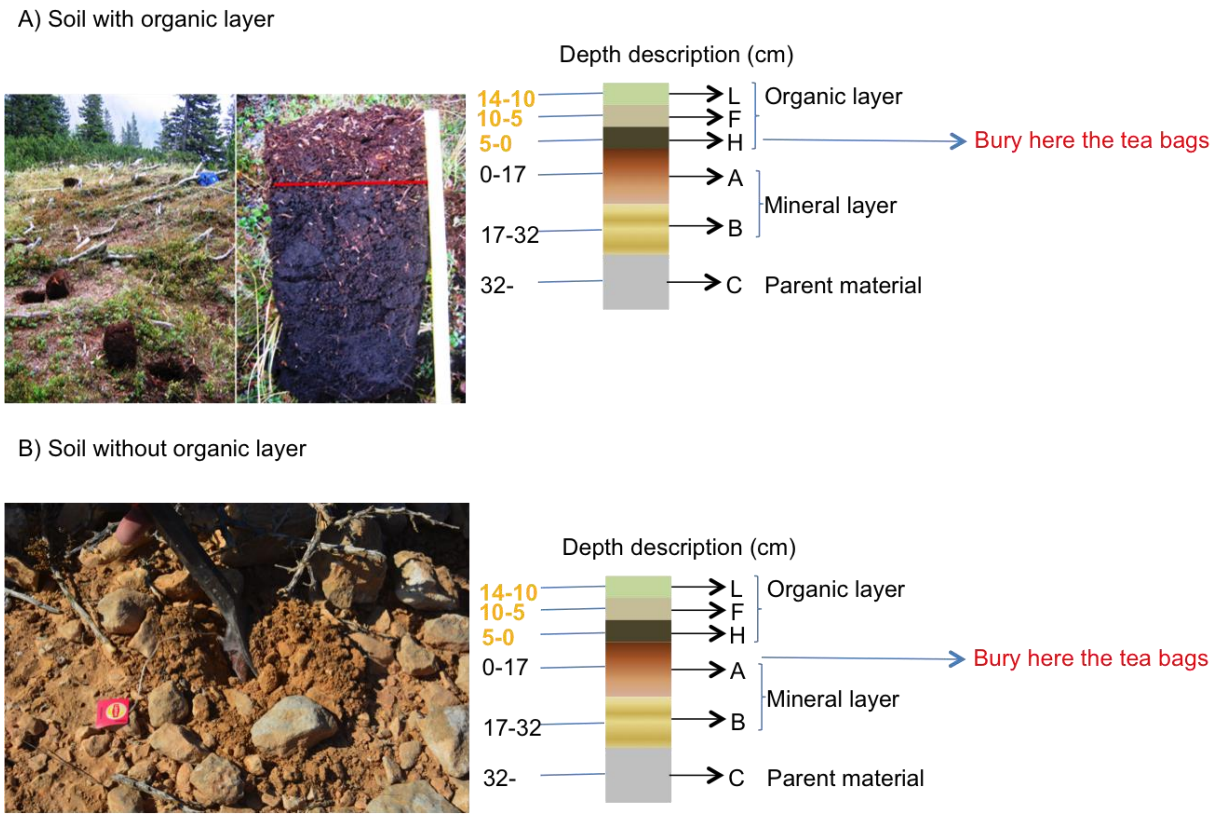

Figure 4: Work steps 2.6. - 3

2.7. Take 3 soil samples (each approx. 100g) from the organic soil layer (H horizon; ~3-0 cm after removing the litter layer; see Fig. 4) at each site for the analysis of the main soil properties- **this is only needed if no soil data are available**. 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. The soil collection can be performed at any sampling point during the 3 years of incubation.

2.8. Start the incubation in June 2016 in the Northern Hemisphere and in December 2016 in the 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 and remember.

2.9. Install 16 teabags of Green tea and 16 tea bags of Rooibos tea in each plot into the organic soil layer (ideally in the upper H layer, Fig. 4). If you have a very shallow soil and almost no organic layer, then insert the bags very close to the surface (H-A layer; 0-3 cm) at all sites. This means that for four plots with increasing species diversity in total 64 bags of green tea and 64 bags of rooibos tea will be needed (see Fig. 5). Indicate in your soil description (step 2.6) the exact instalation depth.

2.10 Choose 4 trees for study within a plot (with approximate 5 m between neighboring trees/ design shall be adapted to fit spatial resources. If tree distance is smaller than 5 m choose whatever is possible in between trees) and install around the tree (close to the tree trunk) 4 green and 4 rooibos tea bags (Fig. 5). Make 4 times a 3cm 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. 4) so that only tag looks out. 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 (Fig.5). 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. 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 stapler with you to attach them again.

2.11. Mark the trees so that you can find them easily. Draw a sketch of the teabag set up (Fig.5).

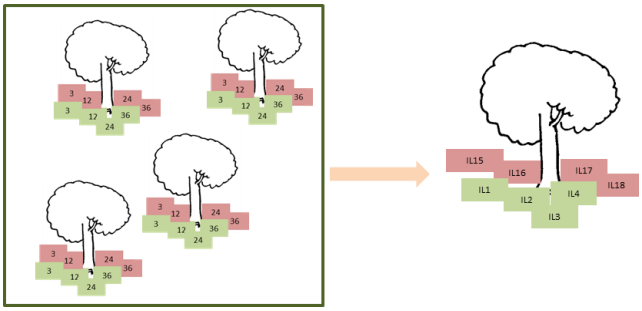

Figure 5: Sketch of the study set up (left: sampling intervals, right: sample order)

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); per plot 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. At the lab:

Figure 6: 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 preferably 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 name/ sample ID/tea type, incubation length, date) and close the glassine bag (Fig. 6). Note if the bag was damaged or found at the surface.

4.5. Use default value (0.248 g) for the empty tea bag
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 (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. 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 minimum data requirement over the incubation period (i.e. June 2016-June 2019) is:

- Daily air temperature for the incubation periods.
- Daily precipitation for the incubation period
- 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: Daily soil temperature (5 cm depth)
- If possible: Daily or monthly soil moisture (5 cm depth)
- Basic soil properties (pH, OC, Ntot, P, S, K, Ca, Mg, Mn) and heavy metals (Cu, Zn, Pb, Cd)- H horizon; ~3-0; only ones during the 3 year of incubation.
- *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 analyzed 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.
- Basically, the samples should be taken but the analyses will depend on local or central funding

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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6. Outputs, benefits, results, 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 (Hypothesis 1) and including the evaluation of the influence of key drivers of change (climate, soil, management, tree species, plant diversity etc.) (Hypothesis 1 and hypothesis 3) on key processes and potentially the relationship of this tea dynamic to local litter quality (Hypothesis 2). In addition, the database will be aligned with similar databases for other corresponding ecosystem networks allowing for global analyses and model applications (Hypothesis 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 announced of the use of their data and with a chance to interfere.

It is foreseen 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, DroughNet 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 inouts 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
- 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

7. Litter bags with native litter

The Teacomposition approach 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 modeling. Therefore, it would be of advantage installing the tea bags together whit the local litterbags or using the sites for the installation with already existing data on native litter decomposition.

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

7.2. Make litterbags 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 (non-corrosive aluminum

tag; Fig.6).

Figure 7: Litterbag

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 they 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).