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Sampling methodology

- *Do you use a reliable, well-developed methodology to investigate vegetation or to answer important questions related to vegetation, which you would like to popularise?*
- *Did you invent a new or unusual approach to study phenomena related to vegetation, which you would like to share?*
- *Do you find certain methods in vegetation science problematic that you would like to discuss and improve?*

If your answer to any of these questions is YES, then you may be an ideal person to contribute to our new forum devoted to methods, newly established as a regular section of the IAVS Bulletin.

Using tea bags to estimate the rate of soil organic matter decomposition in a Taiwanese forest

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When we first began to study changes in forest vegetation along an elevation gradient in Taiwan, from lowland rainforest to mountain cloud forest, one consideration was how to measure the amount of nutrients in the soil. Cloud forests are known to be seriously nutrient-limited, mainly because of lower temperatures and higher relative air humidity, which result in water-saturated soils with slow rates of organic litter decomposition. Slow decomposition means lack of nutrients, where nutrients remain in the soil in the form of undecomposed organic matter (that is why walking in the cloud forests sometimes feels like stepping on pillows full of feathers). We analysed soil samples in the lab for a whole range of chemical compounds, but then we thought – if the lack of nutrients is caused by slow decomposition rate, shouldn't we directly measure decomposition? But how to measure the rate of decomposition in the field, especially if some of our sites needed days of hiking with a heavy pack in steep mountains?

A standard way of measuring decomposition rate is to collect mixed leaf litter from the vegetation under the study, prepare a set of litter bags, bury them in the field, and then in one- or two-week intervals, sample them one by one to build the decomposition curve. For this, we would need to visit each site multiple times in rather short intervals, which was logistically impossible. Then we came across the idea proposed by Keuskamp et al. (2013): replacing the litter bags by commercially available tea bags of two different teas, green tea and rooibos tea. The method sounded promising: we needed to bury teabags in the soil at the beginning, and then pick them up at the end of the decomposition period (around 90 days in our case). In summer 2018 we undertook the pilot study, where, on a subset of localities, we tested whether it actually worked. We found that it did look promising, and decided to give it a proper try. In 2019 we buried teabags on all 18 permanent sites within our elevation transect between 850-2100 m a.s.l., with six pairs of teabags buried in each 20 m x 20 m plot.

How does the method work? First, we actually needed to get the right tea, packed in a standardised way in non-decomposable nylon bags. The study of Keuskamp et al. (2013) was based on commercially available tea manufactured by British company Lipton, which is exported into the whole world. Getting these teabags in Taiwan was not that easy; Taiwanese do not drink rooibos at all, and green tea planted in Taiwanese mountains is much better than the one sold by Lipton (sorry, Lipton, but it is true), so the teabags needed to be imported from elsewhere. Once we got them, the teabags needed to be prepared. We dried them in the dryer to get rid of moisture and weighted each one of them before we sealed them into small plastic bags and prepared for the fieldwork. Teabags from nylon or polypropylene themself are almost identical, but the amount of tea inside may slightly differ among individual bags, that is why the weighting is necessary. In the field, we buried each pair of teabags in approximately 8 cm depth, close to each other (but not in the same place). The string of the tea with the label needs to be visible from outside, so that we could find the teabags again three months later. For sure, we also marked the string with red electrical tape, and recorded the detailed position of each pair of teabags into the map of the permanent plot. When we ran the pilot study, we used four replicates of tea pairs at each site. However, we found that especially in lowland sites, the tea bags got quite often broken – sometimes mechanically (by stones in the soil, by roots passing through), but often also by the soil fauna (ants and beetles seems to love the tea). That is why, in the final study, we used six replicates per plot, which seemed to be sufficient. Three months later, we revisited each site, and carefully dug the teabags out, one by one. It was interesting to see how fast the fine tree roots are growing. When we buried the bags, we had to dig the hole to put them in the soil, but when we want to pick them up, the soil surface was often completely overgrown by fine roots. In rare cases the teabags got broken when we were digging them out, some were broken already in the soil, but in general, we had quite a success. Back in the lab, we dried teabags in the dryer again, weighted the content and compared it with the weight before the experiment. Green tea decomposes much faster then rooibos tea, which is actually why these two need to be buried together. After three months, only a small amount of stabilised recalcitrant part of the green tea biomass remained in the bag, while rooibos still had a considerable amount of labile components. Using modified formulas from Keuskamp et al. (2013) and by averaging the results of unbroken teabags from each site, we were able to calculate the decomposition rate k and stabilisation factor S for each vegetation plot.

Figure 1. (a) New teabags prepared for the experiment. (b) Teabags buried in the soil, with tags visible on the surface. (c) After ca 90 days, teabags were dug out. (d) After drying 48 hours at 70°C, teabags were ready for weighting. (e) Each teabag was opened and the tea remnants weighted. (f) Measuring the weight of decomposed tea. The process of teabag excavation in Taiwanese lowland sub-tropical forest can be also seen on this video: https://vimeo.com/390990611/3ca00eca4f.

As we expected, the decomposition was strongly related to elevation, but also to soil depth and soil pH (see the Fig. 2 for results of analysis where we predicted the decomposition rate by other measured environmental factors in our sites). The decomposition rate is an important contribution to the set of environmental variables we measured in each site, along with other chemical, topographical and microclimatic variables and helps to explain the taxonomical and leaf trait changes in the forest vegetation among our sites.

What next? At the time of writing, we imported a big box of Lipton tea from a Dutch supermarket, and plan to bury them in our 1-ha cloud forest dynamics plot, to evaluate the soil organic matter decomposition on a much finer spatial scale. We also plan to share our data with some of the global initiatives:
http://www.teatime4science.org/,
https://www.teacomposition.org/,
http://www.bluecarbonlab.org/teacomposition-h2o/
to collaborate with other studies and allow comparison of our data with teabag decomposition measurements from other biomes and geographical locations. Some time ago, Lipton changed the type of the material it uses for teabags, from nylon mesh to nonwoven polypropylene (PP) material, which is claimed to be environmentally more friendly. So far it is not clear whether the results from new PP bags will be comparable with original nylon bags, but hopefully so. Indeed, plastic pyramid teabags are not environmentally friendly at all. While used paper teabags can be decomposed in the compost, slowly degrading plastic teabags are not suitable for that, and it is perhaps just a matter of time when the company will switch to other types of materials. Such a move will be environmentally much friendlier, but unfortunately, it will bring an end to this neat, handy decomposition method. Try it while you can!

References

Keuskamp, J.A., Dingemans, B.J.J., Lehtinen, T., Sameel, J.M., & Hefting, M.M. (2013). Tea Bag Index: a novel approach to collect uniform decomposition data across ecosystems. *Methods in Ecology and Evolution*, 4, 1070-1075. https://doi.org/10.1111/2041-210X.12097

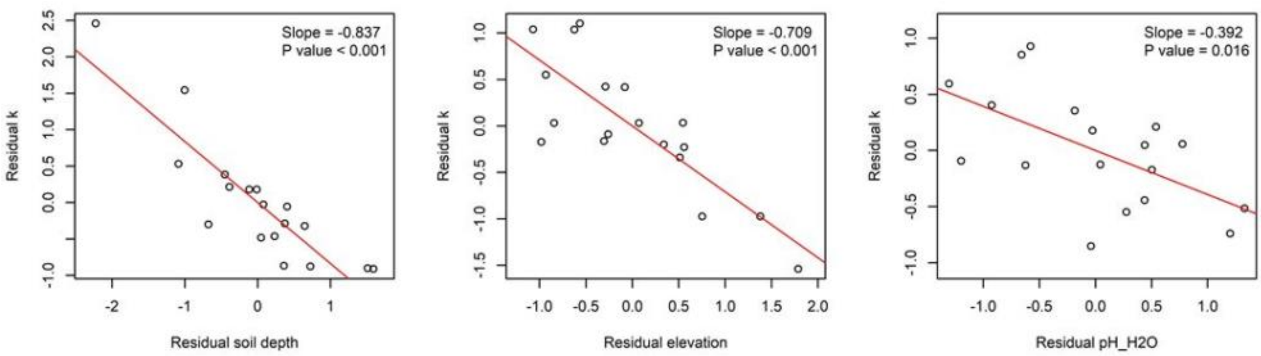

Figure 2. At each site where we measured the decomposition rate using the teabag method, we measured also a set of other relevant environmental variables (soil chemical properties, topography, available light). We were curious to know which of these variables might be good predictors of the decomposition rate *k* measured by teabags. We explored a wide range of different variables and ended up with soil depth, elevation and soil pH (measured in water) as important predictors of decomposition, all of them significantly negatively related to the decomposition rate *k*. Their relationship to *k* is visualized by multiple linear regression (see partial regression diagrams with all variables standardized to zero mean and unit of standard deviation).