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Tea Bag Index: a novel approach to collect uniform decomposition data across ecosystems

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Summary

1. Changes in the balance between soil carbon storage and release can significantly amplify or attenuate global warming. Although a lot of progress has been made in determining potential drivers of carbon release through large-scale decomposition experiments, climate predictions are still hampered by data limitation at a global scale as a result of high effort and measurement costs of comparative litter decomposition studies.

2. We introduce an innovative, cost-effective, well-standardised method to gather data on decomposition rate and litter stabilisation using commercially available tea bags as standardised test kits. By using two tea types with contrasting decomposability, we can construct a decomposition curve using a single measurement in time. The acquired Tea Bag Index (TBI) consists of two parameters describing decomposition rate (k) and litter stabilisation factor (S).

3. The method was tested for its sensitivity and robustness in contrasting ecosystems and biomes, confirming that the TBI is sensitive enough to discriminate between these systems. Within an ecosystem, TBI is responsive to differences in abiotic circumstances such as soil temperature and moisture content. The collected k and S values are in accordance with expectations based on decomposition process literature. They are therefore interpretable within the current knowledge framework.

4. Tea Bag Index is a unique, multifunctional method requiring few resources and minimal prior knowledge. The standardisation and simplicity of the method make it possible to collect comparable, globally distributed data through crowdsourcing. TBI can further provide an excellent decomposition reference and has the potential to increase reliability of soil carbon flux estimates based on extrapolations of decomposition data.

Key-words: climate change, crowdsourcing, field sampling, green tea, litter bag, litter decomposition, microbial ecology, rooibos tea

Introduction

Ecosystem carbon emissions are fundamentally driven by the balance between primary production and respiration, much of which is derived from decomposition of plant litter. The regulating factors of these processes are relatively well studied, but it remains a challenge to separate effects of environmental factors on decomposition from litter quality and litter trait effects. Global climate models generally estimate terrestrial soil respiration on the basis of relationships between climate and map-based soil quality data (Sanchez *et al.* 2009). This method leaves large uncertainties due to the diverse interactions between decomposition and climate driven by changes in CO_2 concentration and temperature. These uncertainties can only be resolved by a more process-based evaluation of decomposition and the related carbon efflux from soils (Heimann & Reichstein 2008).

Earlier efforts to obtain standardised global scale decomposition data made use of different cellulose objects such as cotton strips (Harrison, Latter & Walton 1988; Correll *et al.* 1997; Slocum, Roberts & Mendelssohn 2009). The relation to litter decomposition can be weak as these methods do not account for the complex chemical composition of plant litter, ignoring interactions among the decay of cellulose and other plant constituents (Tiegs *et al.* 2007; Fritz *et al.* 2011).

Only a handful of studies have used plant litter to test decomposition on a global scale (Berg *et al.* 1993; Trofymow *et al.* 2002; Parton *et al.* 2007). They show that the combination of temperature and moisture can explain 50–70% of the variation in decomposition. These studies used coarse grids, sampling 20–39 locations in 1–7 biomes, often not spanning the whole North to South gradient or lacking extreme environ-

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Fig. 1. Tetrahedron-shaped synthetic tea bags used for Tea Bag Index (TBI) experiments.

ments (Berg *et al.* 1993; Trofymow *et al.* 2002; Parton *et al.* 2007). Testing the current generation of climate models with the litter decomposition data obtained from these studies (Bonan *et al.* 2012) revealed that there is a strong need for higher resolution measurements with a global coverage to increase the predictive power of such models (Bonan *et al.* 2012; Stockmann *et al.* 2013).

The approach described here uses a standardised plant litter to measure decomposition and stabilisation at a scale and resolution not previously possible. The key component of the approach is the use of commercially available tea bags (Fig. 1) as highly standardised test kits containing tea as representative dead plant material. Uniquely, this method enables the generation of a global database with the participation of volunteers worldwide. The gathered data can be used to compute a Tea Bag Index (TBI) that provides process-driven information on soil functions at local, regional and global scales. TBI is determined through a simplified litter bag experiment (Wieder & Lang 1982) which involves burial of green and rooibos tea bags, followed by measurement of mass loss after a period of time.

The TBI has two primary applications. First, it is an attainable way to increase the resolution of decomposition measurements. Secondly, TBI is a useful reference alongside decomposition studies to disentangle litter quality aspects from the full set of environmental conditions constituting the 'decomposition matrix'. The use of TBI as a reference facilitates data comparison between biomes, ecosystems and soil types.

Materials and methods

TEA MATERIAL

A simplified litter bag experiment was carried out with commercially available tetrahedron-shaped synthetic tea bags with sides of 5 cm containing *c.* 2 g of green tea or rooibos tea (Lipton, Unilever; Fig. 1). The green tea consisted of 89% green tea, and the rooibos tea consisted of 93% rooibos: both were supplemented with natural flavouring. Mesh size of 0·25 mm allowed microorganisms and mesofauna to enter the bags, but excluded macrofauna (Setälä, Marshall & Trofymow 1996).

CHEMICAL ANALYSES

Green tea and rooibos tea were analysed for carbon fractions using a sequential extraction technique (Ryan, Melillo & Ricca 1990). Four fractions were determined by sequential extraction: nonpolar extractives (NPE), water solubles (WS), acid solubles (AS), and acid insolubles (AIS). The NPE (e.g. fats and waxes) and WS (e.g. simple sugars and phenolics) fractions were continuously extracted for 24 h using a Soxhlet apparatus with dichloromethane followed by deionised water as solvents. Sulphuric acid (72%) was used to extract the AS (e.g. cellulose) fraction. The remaining material [AIS (e.g. lignin) and ash] was combusted at 550°C to determine the ash content. The hydrolysable fraction H is defined as the sum of the NPE, WS and AS fractions (Table 1). H is assumed to be rapidly decomposable in contrast with the recalcitrant nonhydrolysable fraction (AIS and ash).

Total carbon and nitrogen content was measured on the oven-dried (70 °C) ground tea with a CHN-analyser (EA NA 1110; Carlo Erba, Milan, Italy).

Table 1. Results from ANOVAS of quality parameters and weights of four batches of green tea and rooibos tea with different production numbers (N = 3). Asterisks denote significance levels (*P<0·05, **P<0·01, ***P<0·001)

	Green tea			Rooibos tea		
	Mean ± SD	$F(3,8)$	P	Mean± SD	$F(3,8)$	P
Nonpolar extractable fraction (g g^{-1})	0·066 ± 0·003	5·062	0·030*	0·049 ± 0·013	12·950	0·002 **
Water soluble fraction (g g^{-1})	0·493 ± 0·021	0·975	0·451	0·215 ± 0·009	0·418	0·745
Acid soluble fraction (g g^{-1})	0·283 ± 0·017	0·625	0·618	0·289 ± 0·040	2·149	0·172
Acid insoluble fraction (g g^{-1})	0·156 ± 0·009	0·356	0·787	0·444 ± 0·040	1·166	0·381
Mineral fraction (g g^{-1})	0·002 ± 0·0009	7·084	0·012*	0·004 ± 0·0006	3·158	0·086
Hydrolysable fraction (H) (g g^{-1})	0·842 ± 0·023	0·295	0·828	0·552 ± 0·050	1·189	0·374
Total carbon (%)	49·055 ± 0·109	0·243	0·864	50·511 ± 0·286	2·769	0·111
Total nitrogen (%)	4·019 ± 0·049	0·151	0·926	1·185 ± 0·048	0·727	0·564
C : N ratio	12·229 ± 0·129	0·145	0·930	42·870 ± 1·841	0·774	0·541
Total tea bag weight (g)	2·019 ± 0·026	1·260	0·351	2·152 ± 0·013	0·848	0·506
Empty bag weight (g)	0·246 ± 0·001	2·058	0·184	0·245 ± 0·001	0·487	0·701

IN VITRO INCUBATION

To determine decomposition of green and rooibos tea over time, we incubated the tea bags *in vitro* in incubators at 15 °C and 25 °C ($n = 6$), which are well within the expected range of summer soil temperatures. Soil for incubation was collected in spring in a deciduous broadleaf alluvial forest in Landgoed Rhijnauwen, the Netherlands (52°4′11″N, 5°10′35″E). The tea bags were incubated in the dark in covered boxes on a layer of the collected soil underlain by saturated sand to prevent the soil from drying out. After 0, 4, 7, 14, 30, 68 and 130 days of incubation the bags were retrieved, dried (48 h, 70 °C) and weighed. We used the remaining mass to fit exponential decay functions (eqn 2) for both tea types at both 15°C and 25°C.

FIELD APPLICATION

We tested our method in different ecosystems using the protocol described in Box 1. Green and rooibos tea bags were buried pairwise at a depth of 8 cm and retrieved after *c.* 90 days (see Table S1 for location details). We buried between 5 and 32 pairs of tea bags per location. The bags were oven-dried for at least 48 h at 70°C and weighed after removal of adhered soil particles. Burial depth of 8 cm prevented loss or displacement of the bags yet allowed that they were still located in the active soil layer (Schenk & Jackson 2002; Laio, D'Odorico & Ridolfi 2006). Moreover, environmental influences, such as temperature and moisture content, are more stable in the soil than under the litter layer. The mesh size did allow ingrowth of fine roots, but they were easily removable by hand. We did not observe substantial accumulation of roots and fungal biomass in our incubations.

Box 1. TBI protocol

1 Use one bag of Lipton green tea (EAN: 87 22700 05552 5) and one Lipton rooibos tea (EAN: 87 22700 18843 8) per replicate.

a. To obtain better estimates of TBI, bury more replicates per site.

2. Measure the initial weight of the tea bag and subtract the weight of an empty bag (see also Table 1) to determine the initial weight of the tea.

3. Mark the tea bags on the white side of the label with a permanent black marker.

4. Bury the tea bags in 8-cm deep, separate holes while keeping the labels visible above the soil and mark the burial site with a stick.

5. Note the date of burial, geographical position, ecotype and experimental conditions of the site.

6. Recover the tea bags after *c.* 90 days

7. Remove adhered soil particles and dry in a stove for 48 h at 70°C (not warmer!).

8. Remove what is left of the label but leave the string, weigh the bags and subtract the weight of an empty bag without the label to determine the weight after incubation.

a. To get a more precise estimation, open the bag and weigh its content; combust the content at 550°C and subtract what is left from the content weight.

9. Calculate stabilisation factor S and decomposition rate k using eqn 1b.

10. More (facultative) instructions and tips on how to incorporate the TBI in scientific experiments can be found on our website: http://www.decolab.org/tbi

TBI PARAMETERS

In litter bag studies, decomposition is measured by weight loss of plant material in time. A decomposition curve is often estimated by fitting this weight loss to an exponential decay function with decomposition rate constant k. This approximation assumes that half-life of litter is constant in time. The problem with this assumption is that, as decomposition progresses in time, easily degradable compounds in plant litter will be rapidly decomposed, while more recalcitrant compounds will be lost at relatively lower rates. As a result, k is no longer constant as it decreases with time due to the increasing proportion of recalcitrant material.

A simple, but relatively accurate approximation of this process is reached when grouping labile and recalcitrant compounds and estimating k separately for those two groups (Wieder & Lang 1982):

$$W(t) = ae^{-k_1 t} + (1 - a)e^{-k_2 t} \qquad \text{eqn 1a}$$

where $W(t)$ is the weight of the substrate after incubation time t, a is the labile and $1-a$ is the recalcitrant fraction of the litter. The decomposition rate constants of the labile and recalcitrant fractions are described by k_1 and k_2, respectively. During the first phase, the labile fraction is rapidly broken down and the weight loss of the litter is mainly determined by k_1. When all labile material is gone, weight loss is determined by k_2. By definition, k_2 is very low, so that it can only be estimated on very long time scales. To calculate the TBI, we assumed that during short field incubations, the weight loss of the recalcitrant fraction is negligible. As a consequence, k_2 equals zero, and a becomes the decomposable fraction. This reduces eqn 1a to:

$$W(t) = ae^{-kt} + (1 - a) \qquad \text{eqn 1b}$$

Decomposition rate constant k can only be estimated from the early stages of decomposition, while decomposable fraction a, which is conceptually equal to the limit value (Berg & Meentemeyer 2002), is only estimable most of the labile material is gone.

Estimating both k and a would require time series, when only one litter type is used. Instead, we use two litter types with different decomposition rates. The decomposition rate of rooibos tea is low in comparison with green tea. Consequently, decomposition of labile material still continues in rooibos tea after all labile material in green tea has already been consumed. The difference between these litter types allows us to estimate the decomposable fraction from green tea (a_g) and decomposition rate constant k from rooibos tea at a single point in time.

To solve eqn 1b, estimation of the decomposable fraction of rooibos tea (a_r) is needed. We do so by making use of the relation between decomposable fraction a as measured in the field and hydrolysable fraction H, the chemically expected labile fraction. a_r can be estimated from a_g, when assuming that the relation between H and a only depends on environmental conditions.

During decomposition, parts of the labile compounds stabilise and become recalcitrant (Prescott 2010). This stabilisation depends on environmental factors (Berg & Meentemeyer 2002) and results in a deviation of the actual decomposed fraction (i.e. limit value) a from the hydrolysable (i.e. chemically labile) fraction H. This deviation can therefore be interpreted as the inhibiting effect of environmental conditions on the decomposition of the labile fraction and will be referred to as stabilisation factor S:

$$S = 1 - \frac{a_g}{H_g} \qquad \text{eqn 2}$$

where a_g is the decomposable fraction and H_g is the hydrolysable fraction of green tea.

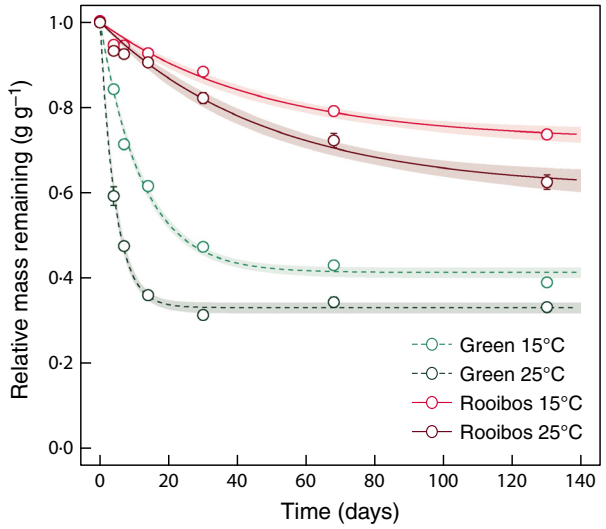

Fig. 2. Relative mass remaining of rooibos and green tea as measured in laboratory incubations on temperate forest soil at 15°C and 25°C. The tea bags were incubated in the dark in covered boxes with moist soil on top of saturated sand and retrieved after 0, 4, 7, 14, 30, 68 and 130 days of incubation ($n = 6$). Lines show fitting to exponential decay function (eqn 2) with 95% confidence intervals. Vertical bars represent standard errors.

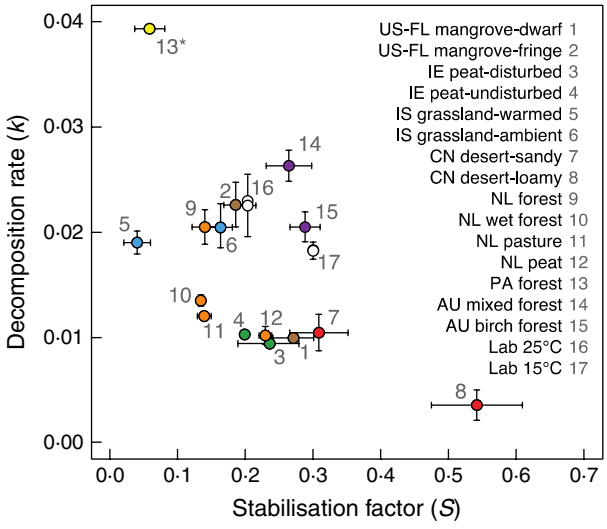

Fig. 3. In situ initial decomposition rate k and stabilisation factor S for different sites showing the discriminatory potential of Tea Bag Index (TBI) between and within ecosystems. k represents short-term dynamics of new input and S is indicative for long-term carbon storage. Calculations were based on a single incubation time between 66 and 90 days. Labels indicate country (United States–Florida (US-FL; $n = 10$), China (CN; $n = 5$), Panama (PA; $n = 20$), the Netherlands (NL; $n = 4$), Austria (AU; $n = 10$), Ireland (IE; $n = 5$) and Iceland (IS; $n = 32$) followed by ecosystem and either soil type or temperature (Table S1). The laboratory incubations shown in Fig. 2 were also included (16–17; $n = 6$). Error bars are standard errors. *Error bars missing due to overdispersion.

The decomposable fraction of rooibos tea (a_r) is calculated from the hydrolysable fraction of rooibos tea (H_r) (Table 1) and the stabilisation factor S:

$$a_r = H_r(1 - S) \qquad \text{eqn 3}$$

With $W_r(t)$ and a_r known, k is calculated using the exponential decay function given in eqn 1b.

The implicit assumption in eqn 3 is that S is equal for both tea types, that is, that the environmental stabilisation of labile material is independent of the relative size and composition of the hydrolysable fraction. To test to what extent the obtained results depend on this assumption, all statistical analyses were repeated under the alternative assumption that stabilisation of hydrolysable rooibos material does not occur, so that S is always zero and $a_r = H_r$. None of the reported relations changed in significance or direction, confirming that the results obtained with the TBI are robust for deviations from the intuitive assumption made in eqn 3 (data not shown).

RELATING TBI PARAMETERS TO ENVIRONMENTAL FACTORS

We related the calculated k and S values obtained from our field sites to temperature and precipitation, which are key environmental factors for decomposition (Prescott 2010). The relation of k and S with temperature was explicitly tested on data from Iceland, where temperature varied considerably (*c.* 12°C) on very short distances due to geothermal activity (Dingemans, unpublished data). S values calculated for the field data set were correlated with classes of carbon sequestration suitability based on soil, climate, moisture and land cover conditions as defined by FAO (2000). Mean annual temperature (MAT) and mean annual precipitation (MAP) were obtained from weather stations closest to the incubation sites (Cantymedia Weatherbase 2013).

In our data set, the MAT and MAP correlated strongly ($r = 0{\cdot}84$, $N = 17$, $P < 0{\cdot}001$) so that we decided to construct a joint climate factor calculated by averaging relative values of MAP and MAT. The relation of k and S with this climate factor and other environmental factors were analysed using ANOVA on linear mixed models with location as a random factor [R package: lme{nlme} (Pinheiro *et al.* 2012)]. The Icelandic sites were excluded from these analyses as their decomposition largely depended on local geothermal conditions. Within the Icelandic site, the relation of k and S with soil temperature was analysed using a linear model. Levene's test was used to test for homogeneity of variance and Shapiro–Wilk test to confirm normality of residuals. All statistical tests were conducted using the R statistical package (R Core Team 2012).

Results and discussion

LABORATORY INCUBATION

Decomposition dynamics of rooibos and green tea were monitored in a laboratory incubation with multiple harvests. Initial decomposition of green tea was very fast, and began to level off after 40–60 days (Fig. 2). Decomposition was much slower in rooibos tea, only starting to level off towards the end of the laboratory incubation experiment. For a large proportion of the incubation time, green tea had already reached its limit value, allowing estimation of S, while the labile fraction of rooibos tea was still actively decomposing, allowing estimation of k. Based on this result, the duration of TBI field incubations was set to 90 days. This period is expected to be sufficiently long to determine stabilisation (S) by measuring the weight loss of the green tea, while short enough to determine initial decomposition rate (k) of the rooibos tea under a wide range of environmental conditions.

GLOBAL APPLICATION

Field application of the TBI found a clear discrimination of both k and S between ecosystems after an incubation period of *c*. 90 days (Fig. 3). Calculated k values increased with mean annual temperature and precipitation ($\chi^2 = 6{\cdot}0$, $P < 0{\cdot}05$) in accordance with general expectations of litter decomposition rates (Parton *et al*. 2007; Zhang *et al*. 2008). k was expected to be higher in geothermally warmed Icelandic plots than in ambient plots, but no significant difference was found.

S values decreased with mean annual temperature and precipitation ($\chi^2 = 6{\cdot}7$, $P < 0{\cdot}01$). We expected S to increase with terrestrial soil carbon sequestration potential as defined by FAO (2000). Indeed this relation was significant ($\chi^2 = 46{\cdot}2$, $P < 0{\cdot}001$): S was low in tropical rainforest (site 13 in Fig. 3), intermediate in forest on humic soils (sites 9 and 10) and high in carbon-accumulating peatlands (sites 3, 4 and 12). A comparison of S between warmed and ambient Icelandic grassland plots (sites 5 and 6) showed that S was lower for warmed plots [$F(1,52) = 35{\cdot}9$, $P < 0{\cdot}001$]. This indicates a positive feedback between diminishing carbon storage and increased temperature, as suggested by (Davidson & Janssens 2006).

The results presented here show that the TBI decomposition parameters are sensitive to ecosystem specific differences and at the same time follow general climatic trends at a global scale. While this data set suffices to validate the method, a much larger data set is required to unravel the exact nature of the relationships between decomposition and environmental factors. We therefore encourage people to collaborate in expanding the data set, leading to robust global information about decomposition. This effort will also help to evaluate the assumptions made in calculating k and S.

In addition to the results shown, we performed pilot studies with an incubation period of 1 year. In many systems, however, substantial amounts of labile material in rooibos tea had been decomposed after a year, leading to inaccurate estimations of k. In the field experiments, the duration of 90 days proved to be sufficient for most sites. However, low microbial activity, such as in Chinese loamy arid soils (Fig. 3, site 8), made the calculation of S unreliable within the set incubation time, so that this site was excluded from statistical analyses. At the other end of the scale, 3 months proved to be the absolute maximum incubation time in the most active site (tropical forest – Fig. 3, site 13), as mass loss of rooibos tea approached its entire labile fraction.

The incubation time in such extreme sites can be adjusted to facilitate calculation of S and k. Incubation time in sites with extremely high k values (e.g. sites with high temperature and precipitation like site 13 in Fig. 3) can be reduced without influencing the result or the comparability of the TBI parameters. In fact, a reduced incubation time is recommended in cases where the weight loss of rooibos tea approaches the limit value, because this may lead to an underestimation of k. Equally, incubation time can be extended in sites with extremely low k values, as in these cases, it is not certain that green tea has reached its limit value, leading to an overestimation of S. Therefore, we recommend to extend the incubation time in sites with low k values in combination with a high S value (e.g. Fig. 3, site 8). Adjustments of the incubation period facilitate the use of the TBI in extreme environments, generating meaningful parameters in extreme cases.

Conclusions

While this method cannot substitute the thoroughness and precision of conventional litter bag methods, TBI considerably reduces the effort necessary to fingerprint local decomposition. The parameters comprising the TBI, k and S, are meaningful integrative estimators to characterise and compare carbon decomposition dynamics between different biomes, ecosystems and soil types.

We foresee a broad application for TBI:

1. By applying it alongside field decomposition experiments as a reference, TBI can provide a contribution in comparing decomposition rates between field experiments in different biomes and ecosystems leading to new insights in global climate effects on decomposition.
2. The simplicity and cost-effectiveness of the method also make it suitable for educational purposes. By involving citizen scientists and schools, the method can increase awareness of a living soil while simultaneously generating numerous data points.
3. Crowdsourcing with the help of social media and research networks will provide decomposition data with a higher resolution and at a larger scale then previously attainable, improving extrapolations of long-term studies over larger areas. We foresee that, with a wide geographical distribution, a validated global soil decomposition map could be assembled.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. Descriptions of incubation sites with coordinates and estimated k and S with standard deviations. Numbers correspond with the legend of Fig. 3.