Plant Soil (2021) 465:613–621
https://doi.org/10.1007/s11104-021-04968-z

METHODS PAPER

# Don't drink it, bury it: comparing decomposition rates with the tea bag index is possible without prior leaching

**Gesche Blume-Werry · Vanessa Di Maurizio · Ilka Beil · Signe Lett · Sarah Schwieger · Juergen Kreyling**

Received: 14 January 2021 / Accepted: 19 April 2021 / Published online: 8 May 2021


**Abstract**
*Purpose* The standardized 'Tea Bag Index' enables comparisons of litter decomposition rates, a key component of carbon cycling, across ecosystems. However, tea 'litter' may leach more than other plant litter, skewing comparisons of decomposition rates between sites with differing moisture conditions. Therefore, some researchers leach tea bags before field incubation. This decreases comparability between studies, and it is unclear if this modification is necessary.
*Methods* We submerged green and rooibos tea bags in water, and measured their leaching losses over time (2 min – 72 h). We also compared leaching of tea to leaf and root litter from other plant species, and finally, compared mass loss of pre-leached and standard tea bags in a fully factorial incubation experiment differing in soil moisture (wet and dry) and soil types (sand and peat).
*Results* Both green and rooibos tea leached strongly, levelling-off at about 40% and 20% mass loss, respectively. Mass loss from leaching was highest in green tea followed by leaves of other plants, then rooibos tea, and finally roots of other plants. When incubated for 4 weeks, both teas showed lower mass loss when they had been pre-leached compared to standard tea bags. However, these differences between standard and pre-leached tea bags were similar in moist vs. dry soils, both in peat and in sand.
*Conclusions* Thus, despite large leaching losses, we conclude that leaching tea bags before field or lab incubation is not necessary to compare decomposition rates between systems, ranging from as much as 5% to 25% soil moisture.

**Keywords** Early-stage decomposition · Decomposition rates · Leaching · Soil moisture · Tea bag index · Teabags

Responsible Editor: Yolima Carrillo.

G. Blume-Werry (✉) · V. Di Maurizio · I. Beil · S. Schwieger · J. Kreyling
Experimental Plant Ecology, Institute of Botany and Landscape Ecology, Greifswald University, Greifswald, Germany
e-mail: gesche.blume-werry@uni-greifswald.de

S. Lett
Department of Biology, Terrestrial Ecology, University of Copenhagen, Copenhagen, Denmark

## Introduction

Soils store more than three times the amount of carbon than what is currently present in the atmosphere (Fischlin et al. 2007). This carbon storage is heavily controlled by decomposition of organic material, and accurately assessing decomposition rates in terrestrial ecosystems is therefore important to predict current and future soil carbon storage. A very common and useful technique for this assessment are litterbags; small mesh bags filled with plant material which are incubated in the field. However, comparisons of study results across local or global scales have historically been difficult due to differences in the plant and bag material used, as well as the general methodology (depth of burial, drying of litter before incubation, mesh size of litter bags, etc.). To facilitate large-scale comparisons between studies Keuskamp et al. (2013) developed the 'Tea Bag Index' which uses commercially available tea bags and a standard protocol. Indeed, the Tea Bag Index has been widely implemented since its introduction both in traditional scientific and crowd-sourced science studies (e.g., Djukic et al. 2018). In addition, tea bags have also increasingly been used as a readily-available standard material for comparisons of decomposition between sites or treatments within the same study and over time (e.g., Elumeeva et al. 2018; Marley et al. 2019; Schwieger et al. 2020).

Despite the many advantages of using tea bags as standard material, there may also be some inherent problems with this method. It seems likely that plants that are used for tea have a particularly high amount of water-soluble compounds to ensure strong flavour. That is, compared to other plants tea might leach (i.e., lose weight due to loss of water-soluble compounds) disproportionally more, especially in soils with high water contents. Differences in decomposition between sites that are driven by precipitation or soil moisture content (Djukic et al. 2018; Petraglia et al. 2019) may thus only be present in material susceptible to high leaching, such as tea, and not in other plant material. If true, this could lead to erroneous conclusions about drivers of decomposition rates between sites. As a solution, it has been suggested to leach tea bags before they are brought out into the field (e.g., Pouyat et al. 2017; Seelen et al. 2019). Yet, to our knowledge, it has not been tested if green and rooibos tea does indeed leach more than other plant material and if those differences could influence results of decomposition rates under different moisture regimes. Undoubtedly, comparability between studies would decrease substantially if the tea bag protocol is adapted to include leaching before incubation in some but not all studies, and at present, it is unclear if this modification is necessary.

Here, we studied if leaching of tea bags before field incubation is needed to meaningfully compare differences in decomposition rates across habitats of differing moisture conditions. Thereto, we (i) constructed leaching curves for both green and rooibos tea to assess at which point leaching levels-off, then (ii) compared leaching of green and rooibos tea to leaf and root material from three other plant species (a tree, a herb, and a grass), and finally, (iii) compared mass loss of pre-leached and non-leached tea bags in a fully factorial incubation experiment differing in soil moisture (wet and dry) and soil types (sand and peat).

## Materials & methods

### Tea- and litterbags

For all tea bags, we used commercially available Lipton tea bags (green *Camellia sinensis*, EAN no.: 8722700 055525, and rooibos tea *Aspalanthus linearis,* EAN no.: 8722700 188,438) made of non-woven polypropylene in a tetrahedron shape. Additional litterbags (nylon, 5 cm × 6 cm, mesh size 0.14 mm) were filled with leaf and root material from the tree *Alnus glutinosa* (L.) Gaertn. (black alder), the herb *Lythrum salicaria* L. (purple loosestrife) and the grass *Deschampsia cespitosa* (L.) Beauv. (tufted hairgrass). *Alnus glutinosa* seedlings were 3 years old at sampling, and were obtained from a local nursery in summer 2018, and individuals of *L. salicaria* and *D. cespitosa* were collected in the field close to Greifswald in autumn of 2018. All species were kept in pots in the common garden at the Institute of Botany and Landscape Ecology at the University of Greifswald, Germany, until harvest in June 2019. Leaves and fine roots (<2 mm diameter) of each species were harvested, washed clean, oven-dried at 70 °C for 48 h, and cut to a maximum size of 1 $cm^2$. For each species, we filled three replicate litterbags with roots and leaves, recorded the exact weight of the material which was always ~0.5 g, and heat-sealed them. We then used these tea and litter bags in the different experiments, see Fig. 1 for a schematic overview. The materials of the commercial green and rooibos tea bags and bags used for additional plant litter bags unfortunately differed due to practical reasons (availability of heat-sealable material for additional bags). However, even though the non-woven Lipton bags appear to have a smaller mesh size, water soluble compounds can leave these bags easily as well (a prerequisite for tea brewing) and we therefore assume no difference in leaching from the bags of different types. This assumption may not apply to assessing decomposition rates in a field setting in which break-down by soil mesofauna is included, but we believe it to be appropriate when assessing differences in leaching through submersion in water. Further, no chemical

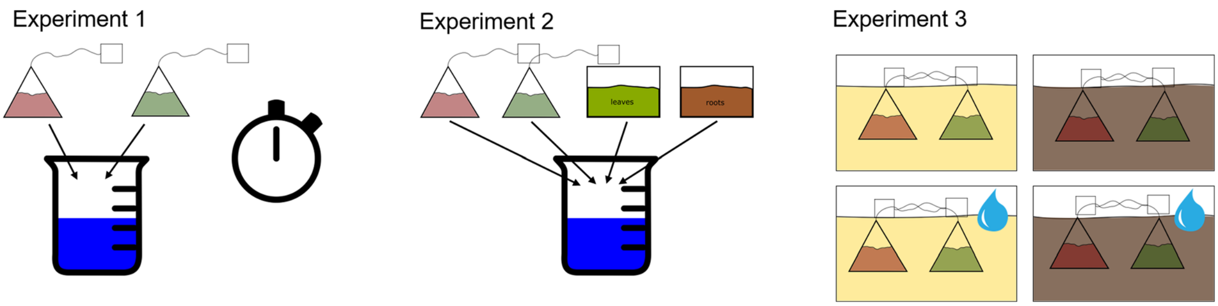


**Fig. 1** Overview of the experiments. In *Experiment 1* we constructed leaching curves for green and roiboos tea over time, in *Experiment 2* we compared leaching between green and roiboos tea, leaves, and roots, and *Experiment 3* was a fully factorial incubation experiment of green and rooibos tea in sand and peat under wet and dry conditions

analyses were done on the leached substrates, so interference from potential differences in of bag material was not an issue in our study.

## Tea leaching curves

We soaked green and rooibos tea bags for varying time intervals to establish a leaching curve showing the relationship between biomass loss and soaking time. We assumed a non-linear relationship between mass loss and time with the strongest differences in the beginning. Thus, we used a gradient design with increasing time intervals and $n = 1$ per time interval, because this set-up outperforms replicated design in detecting and quantifying non-linear responses (Kreyling et al. 2018). In total, we soaked 17 tea bags of both rooibos and green tea, each one in an individual beaker filled with 500 ml of deionized water at a temperature of 25 °C. The time intervals were 2 min, 4 min, 6 min, 10 min, 15 min, 25 min, 35 min, 1 h, 2 h, 3 h, 4 h, 6 h, 12 h, 24 h, 48 h, 69.7 h, and 72.4 h. Afterwards, tea bags were air-dried, oven-dried at 70 °C for 48 h and weighed. A leaching curve of mass loss as a function of leaching time was established following a Michaelis-Menten equation: $y \sim (Vmax*x)/(K_m + x)$; where y is the mass loss (%), Vmax is an asymptote which represents the maximal mass loss of y, x is the leaching time and Km is the Michaelis constant which describes the input (x) that gives 50% of Vmax output.

## Comparison of leaching between green and rooibos tea, leaves, and roots

We compared leaching of green and rooibos tea with leaves and roots from different taxa (see above). All bags were soaked in 500 ml deionized water at a temperature of 25 °C for 12 h, air-dried, oven-dried at 70 °C for 48 h, and weighed. For green and rooibos tea, we used 40 replicate bags for each tea type, for other plant material three replicate bags for each species and plant part (leaves or roots).

## Fully factorial incubation experiment

For our incubation experiment, 40 standard and 40 pre-leached tea bags of each tea type were weighed and buried for 4 weeks (June 24th to July 17th), in separate pots (10 $cm^3$) with one of two different soil types (peat and sand) and two soil moisture conditions (wet and dry), a total of 160 samples. We prepared pre-leached tea bags similarly to the comparison of tea with leaves and roots (see above). Peat soil was standardised bog peat (Torfwerk Moorkultur Ramsloh, Werner Koch GmbH & Co. KG, Saterland, Germany). The peat was not fertilized, but limed to pH 5.6–6.4 at the time of production in May 2019, see Table S2 for more details on the peat substrate. The sand was predominantly medium and fine sand (59% and 21%, respectively, with an additional 11% coarse sand, 4% gravel, and 6% silt and clay). For each combination of treatments, we had ten replicates. Green and rooibos tea that had undergone the same treatment were buried pairwise with a distance of c. 5 cm between them, at a depth of 2 cm in pots. Wet pots were watered to maintain a soil moisture of around 0.25 $m^3/m^3$, dry pots were not watered during the experiment. All pots were randomly placed in bigger boxes in a climate chamber at 25 °C and the position of the boxes was changed at least twice a week. During the first week, soil moisture was measured every day with Decagon Em 50 soil moisture sensors, and in the following weeks, it was measured two to three times a week. Pots in the 'wet' treatment were watered to maintain soil moisture levels. Soil moisture was higher in the wet soil treatments than in the dry soil treatments (wet soils 0.217 ± 0.003 $m^3/m^3$, dry soil 0.052 ± 0.002 $m^3/m^3$, mean ± SE; Fig. S1, Table S1), though wet peat soil (0.247 ± 0.003 $m^3/m^3$) was wetter than wet sand (0.187 ± 0.003 $m^3/m^3$). Soil moisture did not differ between pre-leached and standard tea bags (standard 0.135 ± 0.004 $m^3/m^3$, pre-leached 0.134 ± 0.004 $m^3/m^3$). After 4 weeks, the tea bags were collected, air-dried, cleaned, oven-dried at 70 °C for 48 h, and weighed. We recorded mass loss, and calculated the decomposition rate 'k' and stabilisation rate 'S' according to the Tea Bag Index protocol (Keuskamp et al. 2013).

### Statistical analysis

We pooled observations from the three 'other' plant species into the groups 'leaves' and 'roots', and quantified differences in leaching between leaves, roots, and green and rooibos tea with a linear model and a type III ANOVA (we used type III because of large differences in observations between groups). Differences among groups were subsequently tested using least square means. We quantified the difference between standard and pre-leached tea in each soil type and soil moisture condition with a linear model and type II ANOVA. We performed separate tests for the two tea types, because we were not interested in the large differences between tea types (as tested above). Power analyses were run to test that our experimental design could pick up on small (<5 percentage points) differences in our treatments (see Supporting information for details). All statistical analyses were performed in R (version 3.6.2), and packages used were 'nlme' (Pinheiro et al. 2018) and 'LmerTest' (Kuznetsova et al. 2017) for statistical analysis, 'lsmeans' (Lenth 2016) for posthoc comparison, 'minpack.lm' for fitting the Michaelis-Menten curve (Elzhov et al. 2016), 'Superpower' (Lakens and Caldwell 2021) for power analyses, and 'ggplot2' (Wickham et al. 2016) for visualizations.

## Results

### Tea leaching curves

For both tea types, mass loss rapidly increased with leaching time in the beginning and then levelled off. The fitted Michaelis-Menten equation showed that half of the mass loss from leaching already occurred at 2.4 min and 1.2 min for green ($K_m = 0.04$) and rooibos tea ($K_m = 0.02$), respectively (Fig. 2). Leaching losses then levelled off on a higher level for green ($V_{max}$ = 39.65,) than rooibos tea ($V_{max}$ = 17.36).

### Comparison of leaching between green and rooibos tea, leaves, and roots

Mass loss after 12 h of leaching differed between plant material type ($F = 657.09$, $p < 0.001$), with all types being different from each other (Fig. 3, Tukey PostHoc test: leaves – rooibos tea $p = 0.026$, all other groups $p < 0.001$). Average mass losses were highest for green tea with 41.0%, followed by leaves of other plants with 22.2% (19.6% *A. glutinosa* leaves, 22.2% *D. cespitosa* leaves, 24.7% *L. salicaria* leaves), rooibos tea with 19.4%, and lastly roots with 11.4% (12.8% *A. glutinosa* roots, 15.4% *D. cespitosa* roots, 6.1% *L. salicaria* roots).

### Fully factorial incubation experiment in soil

Pre-leached tea bags showed a much lower mass loss during the incubation experiment than the standard tea bags (43.7% and 62.3% for green tea, and 11.9% and 24.4% for rooibos tea, Table 1). Mass loss was also influenced by soil type, with slightly higher losses in peat than sand, for both green (55.5% in peat, 50.1% in sand) and rooibos tea (21.4% in peat, 14.9% in sand). Soil moisture condition also influenced mass loss with higher mass losses in wet than dry soils (rooibos tea: 20.0% in wet soils, 16.4% in dry soils), albeit with smaller differences than soil type and only marginally significant so for green tea (54.8% in wet soils, 51.2% in dry soils, $P = 0.064$). Only green tea had higher differences in mass loss between pre-leached and standard tea in sand than in peat (significant interaction between pre-leaching treatment and soil type; (Fig. 4, Table 1). The differences in mass loss between soil moisture conditions did depend on soil type as both tea types showed larger differences between wet and dry soil conditions in peat soil than in sand.

However, there was no significant interaction between treatment of the tea bags and soil moisture conditions, despite sufficient statistical power for detecting ecologically meaningful interaction effects (see Supplementary Information). That is, differences in mass loss

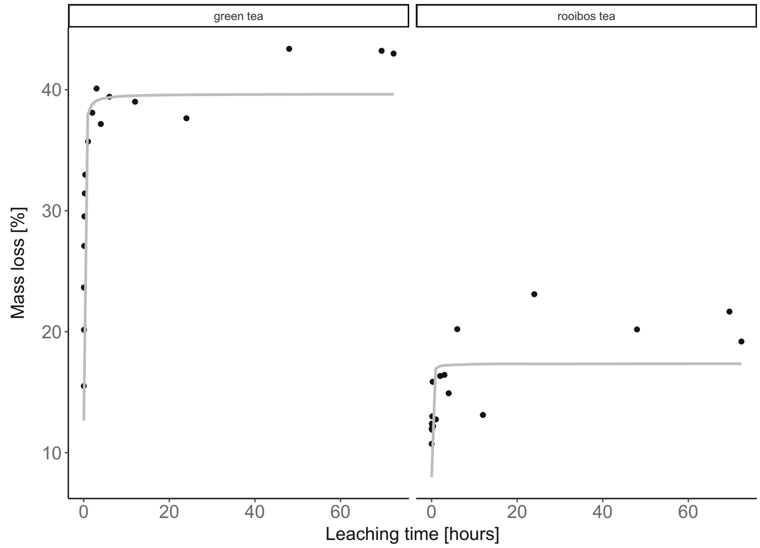


**Fig. 2** Mass loss (%) over time upon inundation in water for green and rooibos tea. A saturation curve following a Michaelis-Menten equation is fitted on the data (grey line), each dot represents one sample, *n* = 17. $V_{max}$ = 39.65, $K_m$ = 0.04 for green tea, $V_{max}$ = 17.36, $K_m$ = 0.02 for rooibos tea

between wet and dry soils did not depend on whether pre-leached or standard tea bags were incubated. There was also no significant three-way interaction between treatment, soil type and soil condition.

The patterns observed for mass loss were the same for the decomposition rate k and the stabilisation factor S (Fig. S2, Table S3). Here, we also found an effect of pre-leaching treatment and soil type, as well as an interaction of soil type and soil moisture condition in both decomposition rate k and stabilisation factor S. Decomposition rates were also slightly higher in wet soils than dry soils, and the stabilisation factor showed larger differences between pre-leached and standard tea bags in sand than in peat (significant interaction between treatment and soil type). But again, neither differences in decomposition rate k nor in stabilisation factor S between wet and dry soils depended on whether pre-leached or standard tea bags were used (no significant interaction between pre-leaching treatment and soil moisture conditions).

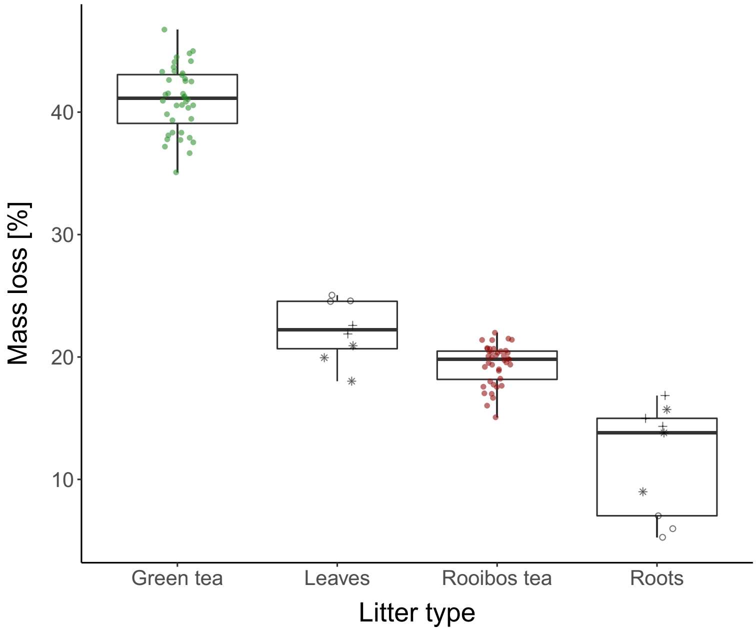


**Fig. 3** Mass loss of green and rooibos tea, as well as leaves and roots (from * *Alnus glutinosa*, ° *Lythrum salicaria*, and + *Deschampsia cespitosa*) after 12 h of leaching. Shown are boxplots with the median, and in which the lower and upper hinges correspond to the 25th and 75th percentiles, and the whiskers extend from the hinges to the values closer than 1.5 × the inter-quartile range, *n* = 40 for green and rooibos tea, *n* = 3 for leaves and roots. Each symbol represents one sample

**Table 1** Effect of treatment, soil type, soil condition, and their interactions on mass loss after 4 weeks' incubation of a) green and b) rooibos tea. Significant effects ($p < 0.05$) are highlighted in bold

| | df | Sum Sq | F value | *P* value |
|---|---|---|---|---|
| (a) Green tea | | | | |
| **Treatment (standard or pre-leached tea bags)** | **1** | **6901.9** | **93.8** | **<0.001** |
| **Soil type (peat or sand)** | **1** | **494.6** | **6.7** | **0.012** |
| Soil condition (wet or dry) | 1 | 260.8 | 3.5 | 0.064 |
| **Treatment: Soil type** | **1** | **357.7** | **4.9** | **0.031** |
| Treatment: Soil condition | 1 | 2.4 | 0.0 | 0.857 |
| **Soil type: Soil condition** | **1** | **819.8** | **11.1** | **0.001** |
| Treatment: Soil type: Soil condition | 1 | 9.8 | 0.1 | 0.717 |
| Residuals | 72 | 5295.2 | | |
| (b) Rooibos tea | | | | |
| | df | Sum Sq | F value | P value |
| **Treatment** | **1** | **3103.0** | **336.4** | **<0.001** |
| **Soil type** | **1** | **869.3** | **94.3** | **<0.001** |
| **Soil condition** | **1** | **280.7** | **30.4** | **<0.001** |
| Treatment: Soil type | 1 | 6.2 | 0.7 | 0.416 |
| Treatment: Soil condition | 1 | 1.0 | 0.1 | 0.746 |
| **Soil type: Soil condition** | **1** | **281.0** | **30.5** | **<0.001** |
| Treatment: Soil type: Soil condition | 1 | 36.1 | 3.9 | 0.052 |
| Residuals | 71 | 654.8 | | |

## Discussion

We used a suite of experiments to explore if pre-leaching, 'brewing', of tea bags is necessary before incubation to accurately assess differences in decomposition between sites or ecosystems that differ in soil moisture. As expected, both tea types did leach substantially upon submergence in water. Nevertheless, while

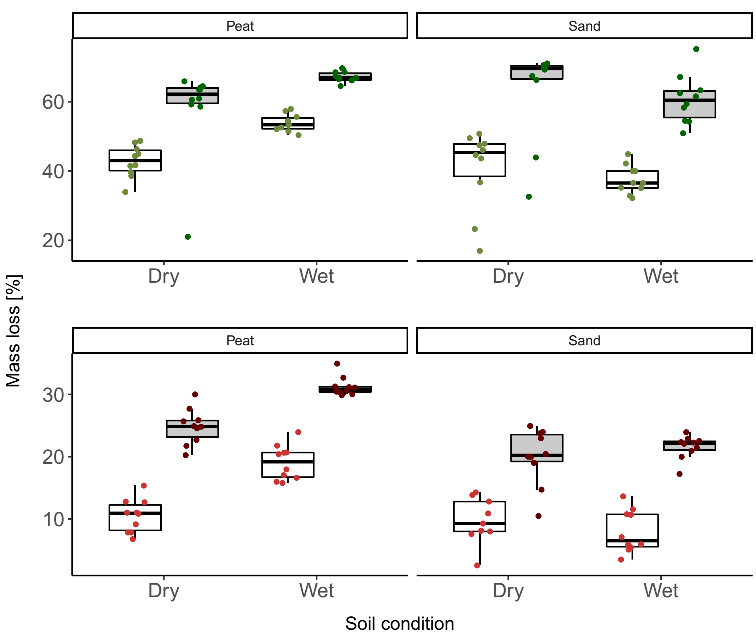


**Fig. 4** Mass loss of green tea (upper panel) and rooibos (lower panel) tea under dry and wet soil conditions in peat and sandy soil, incubated for 4 weeks. Light dots and white boxplots signify pre-leached tea bags, and dark dots and grey boxplots standard tea bags, note that y-axes differ between tea types. Shown are boxplots with the median, and in which the lower and upper hinges correspond to the 25th and 75th percentiles, and the whiskers extend from the hinges to the values closer than 1.5 × the inter-quartile range, each dot represents one sample, $n = 10$ except for rooibos tea in dry sand where $n = 9$

green tea did leach almost twice as much as leaves and almost four times as much as roots, leaching losses from rooibos tea were broadly similar to other leaf material. We also showed that despite lower mass losses when tea bags were pre-leached, the relative differences were similar between wetter and drier soil conditions.

## Tea leaches quickly and substantially, but other plants do too

Both tea types showed a fast and substantial mass loss in response to water inundation. This is not surprising, as Keuskamp et al. (2013) measured 49 and 22% water-soluble fractions in green and rooibos tea, respectively. The mass losses observed here, 41% for green tea and 19% for rooibos tea, were also similar to those observed by other researchers upon soaking the tea bags in hot water (40 and 27%; Pouyat et al. 2017), cooking them for several minutes (31% and 17%; Djukic et al. 2018), or leaching tea from a different brand in cold water (41 and 18% mass loss, Marley et al. 2019). These high losses in response to simple inundation in water observed here and previously reported, led us to believe that results in larger-scale comparisons of decomposition rates may be skewed if habitats differing in soil moisture were included. For example, Djukic et al. (2018) showed using tea bags that temperature did not affect mass loss in a comparison across 336 sites (ranging from −9 to +26 °C MAT and from 60 to 3113 mm MAP), whereas higher precipitation led to higher mass losses. This higher mass loss could be attributed to an increased activity of decomposers with higher moisture availability (García-Palacios et al. 2016), and thus represent a pattern that would be similar if another standard material or local plant material had been used. However, if tea plants leach disproportionally more than other plants, these results would not reflect microbial activity but passive leaching and other plants would show different patterns.

When we compared leaching losses of tea to leaves and roots of three temperate plant species (*A. glutinosa*, *L. salicaria,* and *D. cespitosa*), we saw that while green tea did leach about double the amount, rooibos tea leached on average slightly less than the leaves of the selected plants. This underlines that tea is indeed suitable as a proxy for other plant material (Didion et al. 2016; Duddigan et al. 2020). Though the focus of decomposition studies generally is on leaf litter, we want to point out that root litter did leach substantially less in our experiment than plant leaf litter (including both tea types). In many ecosystems, such as shrublands, grasslands, or tundra, belowground biomass exceeds aboveground biomass (Mokany et al. 2006), highlighting the important role that root litter may have in ecosystem carbon fluxes and storage. This does caution against the representation of tea as root litter, which should be kept in mind when following the instructions of the Tea Bag Index and burying the tea bags at 8 cm depth (Keuskamp et al. 2013).

## Mass losses from pre-leached teas are lower than standard tea bags but unaffected by soil moisture

As expected, mass losses were lower from pre-leached than standard tea bags in our incubation experiment, most likely because they had already lost most water-soluble components before the start of the experiment. Rooibos tea also showed lower mass losses in drier soils. However, to our surprise, the differences between pre-leached and standard tea bags were similar in moist and dry soils, despite sufficient statistical power to detect meaningful interactions (power analysis in the Supplementary Information). That is, the difference in % mass loss between standard and pre-leached tea bags was, for green tea, 18.9 in dry soils and 18.2 in wet soils; and for rooibos tea 12.3 in dry soils and 12.9 in wet soils.

For green tea, the differences in mass loss between pre-leached and standard tea bags were higher in sand than in peat. This could be due to lower microbial activity in the sandy substrate (indicated by generally lower mass losses in sand) leading to a slightly more obvious leaching signal. This is also in line with the lack of this interaction in rooibos tea, which generally showed lower leaching losses. The superpower of the Tea Bag Index is to use readily-available standard material that allows comparisons from experiments around the world (e.g., Djukic et al. 2018), even implemented by crowd-sourced science. However, this advantage of comparability quickly degrades if different pre-treatments are introduced. Our results show that, at least in the broadly different soil moisture regimes tested here, the aims of the Tea Bag Index to serve as a standard material with which environmental influences on decomposition can be compared across sites are not compromised.

## Conclusions

Based on our results, we conclude that tea bags do not have to be leached before incubation. It is very likely that much of the mass loss that is typically observed over short time periods is indeed due to leaching losses rather than microbial mineralization (Djukic et al. 2018; Elumeeva et al. 2018; MacDonald et al. 2018; Marley et al. 2019; Petraglia et al. 2019). Nevertheless, as leaching is part of the normal decomposition process that all plant litter is experiencing (Krishna and Mohan 2017), this does most likely not introduce erroneous conclusions about decomposition rates in terrestrial ecosystems (with the possible exception of roots, see above). We thus urge the ecological community to continue to follow the original tea bag protocol to allow further global comparisons of decomposition, while at the same time being aware that much of the mass loss in the early stages of decomposition is not due to microbial mineralization of the material but rather passive leaching of water-soluble compounds, as is present in most plant material.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11104-021-04968-z.

**Acknowledgements** This study was supported by the European Social Fund (ESF) and the Ministry of Education, Science, and Culture of Mecklenburg-Western Pomerania with the project WETSCAPES (ESF/14-BM-A55-0035/16). VDM was awarded a DAAD RISE (Research Internship in Science and Engineering) stipend, during which the experiment was conducted. We thank the whole Experimental Plant Ecology group for discussions on tea bags.

**Availability of data and material** Data and scripts are available on figshare (doi: https://doi.org/10.6084/m9.figshare.14269268).

**Code availability** See above.

**Author contributions** GBW, IB, SS, and JK conceived the ideas and designed methodology; VDM collected the data; VDM and GBW analysed the data; SL led the power analysis; GBW led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

**Funding** Open Access funding enabled and organized by Projekt DEAL.

**Declarations**

**Conflict of interest** The authors have no conflicts of interest to declare that are relevant to the content of this article.

**Ethics approval** Not applicable.

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

## References

Didion M, Repo A, Liski J, Forsius M, Bierbaumer M, Djukic I (2016) Towards harmonizing leaf litter decomposition studies using standard tea bags – a field study and model application. Forests 7:167

Djukic I et al (2018) Early stage litter decomposition across biomes. Sci Total Environ 628–629:1369–1394

Duddigan S, Shaw LJ, Alexander PD, Collins CD (2020) Chemical underpinning of the tea bag index: an examination of the decomposition of tea leaves. Appl Environ Soil Sci 2020:1–8

Elumeeva TG, Onipchenko VG, Akhmetzhanova AA, Makarov MI, Keuskamp JA (2018) Stabilization versus decomposition in alpine ecosystems of the Northwestern Caucasus: the results of a tea bag burial experiment. J Mt Sci 15:1633–1641

Elzhov TV, Mullen KM, Spiess A-N, Bolker B (2016) minpack.lm: R Interface to the Levenberg-Marquardt Nonlinear Least-Squares Algorithm Found in MINPACK, Plus Support for Bounds. R package version 1.2-1. https://CRAN.R-project.org/package=minpack.lm

Fischlin A, Midgley GF, Price JT, Leemans R, Gopal B, Turley C, Rounsevell MDA, Dube OP, Tarazona J, Velichk AA (2007) Ecosystems, their properties, goods, and services. In: Parry ML, Canziani OF, Palutikof JP, van der Linden PJ, Hanson CE (eds) Climate change 2007: impacts, adaptation and vulnerability. Contribution of working group II to the fourth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, pp 211–272

García-Palacios P, Shaw EA, Wall DH, Hättenschwiler S (2016) Temporal dynamics of biotic and abiotic drivers of litter decomposition. Ecol Lett 19:554–563

Keuskamp JA, Dingemans BJJ, Lehtinen T, Sarneel JM, Hefting MM (2013) Tea bag index: a novel approach to collect uniform decomposition data across ecosystems. Methods Ecol Evol 4:1070–1075

Kreyling J, Schweiger AH, Bahn M, Ineson P, Migliavacca M, Morel-Journel T, Christiansen JR, Schtickzelle N, Larsen KS (2018) To replicate, or not to replicate – that is the question: how to tackle nonlinear responses in ecological experiments. Ecol Lett 21:1629–1638

Krishna MP, Mohan M (2017) Litter decomposition in forest ecosystems: a review. Energy Ecol Environ 2:236–249

Kuznetsova A, Brockhoff PB, Christensen RHB (2017) **lmerTest** package: tests in linear mixed effects models. J Stat Softw 82:1

Lakens D, Caldwell AR (2021) Simulation-based power analysis for factorial analysis of variance designs. Advances in

Methods and Practices in Psychol Sci 4:1–14. https://doi.org/10.1177/2515245920951503

Lenth RV (2016) Least-squares means: the R package lsmeans. J Stat Softw 69:1–33

MacDonald, E., M. E. Brummell, A. Bieniada, J. Elliott, A. Engering, T.-L. Gauthier, S. Saraswati, S. Touchette, L. Turmel-Courchesne, and M. Strack. 2018. Using the tea bag index to characterize decomposition rates in restored peatlands Boreal Environ Res 23:16

Marley ARG, Smeaton C, Austin WEN (2019) An assessment of the tea bag index method as a proxy for organic matter decomposition in intertidal environments. J Geophys Res Biogeosci 124:2991–3004

Mokany K, Raison RJ, Prokushkin AS (2006) Critical analysis of root: shoot ratios in terrestrial biomes. Glob Change Biol 12:84–96

Petraglia A, Cacciatori C, Chelli S, Fenu G, Calderisi G, Gargano D, Abeli T, Orsenigo S, Carbognani M (2019) Litter decomposition: effects of temperature driven by soil moisture and vegetation type. Plant Soil 435:187–200

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2019) _nlme: Linear and Nonlinear Mixed Effects Models_. R package version 3.1-142

Pouyat, R. V., H. Setälä, K. Szlavecz, I. D. Yesilonis, S. Cilliers, E. Hornung, S. Yarwood, D. J. Kotze, M. Dombos, M. P. McGuire, and T. H. Whitlow. 2017. Introducing GLUSEEN: a new open access and experimental network in urban soil ecology. J Urban Ecol 3, https://doi.org/10.1093/jue/jux002

Schwieger S, Kreyling J, Couwenberg J et al (2020) Wetter is better: rewetting of Minerotrophic Peatlands increases plant production and moves them towards carbon sinks in a dry year. Ecosystems. https://doi.org/10.1007/s10021-020-00570-z

Seelen LMS, Flaim G, Keuskamp J, Teurlincx S, Arias Font R, Tolunay D, Fránková M, Šumberová K, Temponeras M, Lenhardt M, Jennings E, de Senerpont Domis LN (2019) An affordable and reliable assessment of aquatic decomposition: tailoring the tea bag index to surface waters. Water Res 151:31–43

Wickham H (2016) ggplot2: Elegant graphics for data analysis. Springer-Verlag, New York