

**Don't throw the baby out with the (leached) bathwater; a reply to
Lind et al 2022**

Authors

Judith M. Sarneel^{1*}, Janna M. Barel², Sarah Duddigan³, J.A. Keuskamp^{4,5}, Ada Pastor Oliveras⁶, Taru Sandén⁷, Gesche Blume-Werry¹

¹ Department of Ecology and Environmental science, Umeå University, SE-901 87 Umeå, Sweden

² Aquatic Ecology & Environmental Biology, Radboud Institute for Biological and Environmental Sciences, Faculty of Science, Radboud University Nijmegen, AJ 6525 Nijmegen, The Netherlands.

³ Soil Research Centre and Department of Geography & Environmental Science, University of Reading, Reading RG6 6UR, UK

⁴ Ecology & Biodiversity group, Institute of Environmental Biology, Utrecht University, Utrecht, The Netherlands

⁵ Biont Research, Utrecht, The Netherlands

⁶ Institute of Aquatic Ecology, University of Girona, Spain

⁷ Department for Soil Health and Plant Nutrition, Austrian Agency for Health and Food Safety (AGES), Vienna, Austria

*Corresponding author: J.M. Sarneel Judith.sarneel@UMU.se

22 **Abstract**

23 During litter decomposition, part of the water-soluble components of the material dissolve
24 (leach) rapidly into available water in the environment. Studies on litter decomposition that
25 quantify mass-loss from litterbags integrate leaching and mineralization. In contrast to Lind et
26 al. (2022), we believe that correcting for leaching in (terrestrial) litterbags studies such as the
27 Tea Bag Index will result in more uncertainties than it resolves. This is mainly because leaching
28 is a continuous process and because leached material can still be mineralized after leaching.
29 Further, amount of material that potentially leaches from tea is comparable to other litter types.
30 When correcting for leaching, it is key to be specific about the employed method, just like being
31 specific about the study specific definition of decomposition.

32

33 **Keywords:** Decomposition, Leaching, Litter mass loss, Mineralization, Tea Bag Index, Reply

Introduction

During litter decomposition, a fraction of the water-soluble components of the litter is quickly dissolved (leached) into the water that is available in the environment. Besides leaching, litter decomposition is driven by fragmentation, (UV)-bleaching and microbial activity. Many studies quantify litter decomposition by measuring mass-loss rates of incubated leaves, which inherently integrate the biotic and abiotic processes that drive litter decomposition. In 2013, the Tea Bag Index (TBI) was published, which is an easy method that uses tea bags as equivalent to litter bags filled with local litter (Keuskamp et al., 2013). Because the plant material used as litter in the TBI has a leaching product we are all familiar with, tea, it inspired Lind et al. (2022) and others (Figure 1a) to explicitly address and quantify leaching. In addition, frameworks like the Microbial Efficiency-Matrix Stabilization (Cotrufo et al., 2013) and increased interest in fluxes of dissolved organic matter from soils (Cleveland et al., 2004) further highlight the role of leaching during litter decomposition. Mechanistic studies such as presented by Lind et al. (2022) contribute to an increased understanding of the factors that drive leaching losses during litter decomposition. However, we disagree with the conclusion Lind et al. (2022) draw from their studies; that there is a need to introduce a leaching correction in the Tea Bag Index. We believe that correcting for leaching (especially in terrestrial TBI and other mass-loss based studies) introduces more uncertainties than it solves. As a result, correcting for leaching hampers the interpretation, decreases comparability across studies and increases the complexity of the TBI that is designed to be a simple method.

Definitions

Litter decomposition is often used as an umbrella concept and its definition may vary due to the aim of the study (Benfield et al., 2017). The narrower definition that places the biological activity in the centre is for instance used when studying the diversity of decomposing organisms (e.g. in Gessner et al., 2010). Mineralization is frequently used as an alternative for this narrower definition (Benfield et al., 2017). Studies that measure mass-loss frequently discuss the role of fragmentation, leaching, bleaching and biological degradation and hence

implicit consensus on a wider definition exists within this field. In this commentary, we use the wider definition and agree with Lind et al. (2022) and Benfield et al. (2017) to be explicit about definitions in order to minimize confusion in the scientific discussion. Moreover, there are two common approaches to account for leaching in mass loss studies, which both may have their own implications. The first is a *a posteriori* mathematical correction of the initial weight based on a local measurement of the weight loss during a short period of time (Lind et al., 2022; Seelen et al., 2019). Alternatively, litterbags are soaked before incubation to remove most of the water-soluble material (in TBI; Blume-Werry et al., 2021; Kotze & Setälä, 2022; Toth et al., 2018; Toth et al., 2017). In this comment, we will focus on the *a posteriori* correction.

Leaching in tea

The TBI consists of burying two types of tea bags as an easy alternative for litter bags filled with local litter (Keuskamp et al., 2013). The mass loss after ca. three months is used to parameterize the litter decomposition curve and obtain a litter decomposition rate that estimates the decay of the soluble and hydrolysable compounds in rooibos tea. Although we do not claim that the tea used in TBI completely represents local litter material, the water-soluble fraction of tea (the total of leachable material) is well in range with other litter (Figure 1b; Harmon, 2016). We therefore disagree with the statement of Lind et al. (2022) that '*initial leaching of water-soluble compounds may therefore be even higher in the tea bag decomposition substrates than for intact leaves of traditional litterbag studies*'. Moreover, leaching measurements by Lind et al (2022) include extremes when compared to other leaching measurements in tea. On average, leaching in rooibos and green tea is within the ranges reported in the three review studies to our knowledge available on leaching (mass loss of 14- 40%, 5.7 - 47.2% and 7-31%, respectively; Friesen et al., 2018; Jiang et al., 2016; Xiong & Nilsson, 1997; Figure 1).

Reasons against a leaching correction

Lind et al. (2022) advocate that correcting litter decomposition rates for leaching would improve the TBI method (and implicitly other litterbag studies). The TBI method intends to obtain a standardized, easy measurement of mass losses and introducing a leaching correction would complicate both its practical use as well its' interpretation. Hence, such correction would throw the baby out with the (leached) bathwater. Specifically, we believe that a leaching correction would introduce a number of uncertainties: Firstly, leaching is a continuous process as both starting products and products resulting from degradation can be leached when environmental conditions allow (Wang et al., 2021). This implies that rain events, or (as Lind et al. (2022 show) temperature changes may cause additional leaching (Wang et al., 2021). Even when the aim is to only correct for initial leaching of the fresh litter, the timeframe in which mass loss is uniquely due to leaching will remain an educated guess. Moreover, initial leaching duration may differ between ecosystems, between seasons within the same ecosystem, due to variation in temperature and water availability and unpredictable precipitation events (Lind et al., 2022). That this is uncertainty is felt by the researching community is reflected by the variable duration of leaching measurements that are applied: For instance, leaching measurements of tea were conducted from 3 minutes to 48 hours (Figure 1). Other than in terrestrial systems, leaching as an initial event is possible to quantify in aquatic systems (Elwood et al., 1981; Gessner et al., 1999; Seelen et al., 2019), but also in this systems, mass-loss studies frequently do not use a leaching correction but integrate all processes that cause litter material to disintegrate (Benfield et al., 2017).

A second uncertainty introduced by the proposed leaching correction is that the leached material is not necessarily exempt from further microbial decomposition. In fact, a large part of the leached components will be mineralized after leaching (Cleveland et al., 2004), although the discussion on which part exactly is not resolved (Cotrufo et al., 2013). Thirdly, when correcting for leaching, one has to consider that its variation due to environmental conditions may induce unknown variation in the starting material. That is, a leaching correction assumes that the leached material is no longer part of the litter, which inevitably means

changes in chemical composition and/or stoichiometry (Schreeg et al., 2013). This, in turn introduces a variation that is hard to quantify and will hamper comparisons, especially given the unstandardized way to measure leaching (Figure 1).

Lastly, Lind et al. (2022) convincingly show that leaching depends on specific settings of the environment. This questions the use of leaching measurements from one location or time point (because temperature and moisture changes over time) to correct mass loss at another location (as in Lind et al., 2022; Seelen et al., 2019). If there is a conceptual and practical need for a leaching correction, this should be done under exactly the same setting as the incubation (Lind et al., 2022; Wang et al., 2021).

Conclusion

Lind et al. (2022) convincingly show that the same factors (temperature and moisture) that affect mineralization can also drive differences in leaching, and flag for higher appreciation of this process in the TBI, mass-loss and litterbag studies. Yet, making a mathematical correction of leaching part of the standardized TBI method is not feasible or desirable. It introduces more uncertainties than it solves and undermines the purpose of the method: standardisation between studies. The TBI was designed to be an easy and reproduceable way to study litter mass loss, by both professional scientists and citizen scientists. TBI, like many other litter bag studies, includes the environmental effects on fragmentation, leaching, bleaching and mineralization. Tea bags could potentially help to disentangle the environmental variables that drive leaching. Future litter decomposition and leaching studies will improve by careful interpretation of solid experiments, being transparent about definitions used and explaining the way in which leaching corrections were applied. Comparison across studies is further enhanced by standardization of the methods used, and as outlined above, a correction for leaching is not advised in TBI.

References

- Benfield, E. F., Fritz, K. M., & Tiegs, S. D. (2017). *Leaf-Litter Breakdown* (3 ed. Vol. 2: Ecosystem Function). London: Academic Press.
- Blume-Werry, G., Di Maurizio, V., Beil, I., Lett, S., Schwieger, S., & Kreyling, J. (2021). Don't drink it, bury it: comparing decomposition rates with the tea bag index is possible without prior leaching. *Plant and Soil*, 465(1-2), 613-621. doi:10.1007/s11104-021-04968-z
- Cleveland, C. C., Neff, J. C., Townsend, A. R., & Hood, E. (2004). Composition, dynamics, and fate of leached dissolved organic matter in terrestrial ecosystems: Results from a decomposition experiment. *Ecosystems*, 7(3), 275-285. doi:10.1007/s10021-003-0236-7
- Cotrufo, M. F., Wallenstein, M. D., Boot, C. M., Denef, K., & Paul, E. (2013). The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? *Global Change Biology*, 19(4), 988-995. doi:10.1111/gcb.12113
- Djukic, I., Kepfer-Rojas, S., Schmidt, I. K., Larsen, K. S., Beier, C., Berg, B., . . . TeaComposition. (2018). Early stage litter decomposition across biomes. *Science of the Total Environment*, 628-629, 1369-1394. doi:10.1016/j.scitotenv.2018.01.012
- Elwood, J. W., Newbold, J. D., Trimble, A. F., & Stark, R. W. (1981). The limiting role of phosphorous in a woodland stream ecosystem - Effects of P-enrichment on leaf decomposition and primary producers. *Ecology*, 62(1), 146-158. doi:10.2307/1936678
- Friesen, S. D., Dunn, C., & Freeman, C. (2018). Decomposition as a regulator of carbon accretion in mangroves: a review. *Ecological Engineering*, 114, 173-178. doi:10.1016/j.ecoleng.2017.06.069
- Gessner, M. O., Chauvet, E., & Dobson, M. (1999). A perspective on leaf litter breakdown in streams. *Oikos*, 85(2), 377-384. doi:10.2307/3546505

168 Gessner, M. O., Swan, C. M., Dang, C. K., McKie, B. G., Bardgett, R. D., Wall, D. H., &
169 Hattenschwiler, S. (2010). Diversity meets decomposition. *Trends in Ecology &*
170 *Evolution*, 25(6), 372-380. doi:10.1016/j.tree.2010.01.010

171 Harmon, M. E. (2016). *LTER Intersite Fine Litter Decomposition Experiment (LIDET), 1990 to*
172 *2002 version 11.*

173 Jiang, L. P., Yue, K., Yang, Y. L., & Wu, Q. G. (2016). Leaching and Freeze-Thaw Events
174 Contribute to Litter Decomposition - A Review. *Sains Malaysiana*, 45(7), 1041-1047.

175 Keuskamp, J. A., Dingemans, B. J. J., Lehtinen, T., Sarneel, J. M., & Hefting, M. M. (2013).
176 Tea Bag Index: a novel approach to collect uniform decomposition data across
177 ecosystems. *Methods in Ecology and Evolution*, 4(11), 1070-1075. doi:10.1111/2041-
178 210x.12097

179 Lind, L., Harbicht, A., Bergman, E., Edwartz, J., & Eckstein, R. L. (2022). Effects of initial
180 leaching for estimates of mass loss and microbial decomposition-Call for an increased
181 nuance. *Ecology and Evolution*, 12(8), 10. doi:10.1002/ece3.9118

182 Madaschi, C., & Diaz-Villanueva, V. (2021). A Warm Tea: The Role of Temperature and
183 Hydroperiod on Litter Decomposition in Temporary Wetlands. *Ecosystems*.
184 doi:10.1007/s10021-021-00724-7

185 Mori, T., Aoyag, R., Taga, H., & Sakai, H. (2021). Effects of Water Content and Mesh Size on
186 Tea Bag Decomposition. *Ecologies*, 2, 175–186. doi:doi.org/10.3390

187 Pouyat, R. V., Setälä, H., Szlavecz, K., Yesilonis, I. D., Cilliers, S., Hornung, E., . . . T.H., W.
188 (2017). Introducing GLUSEEN: a new open access and experimental network in urban
189 soil ecology. *Journal of Urban Ecology*, 3(1), jux002. doi:10.1093/jue/jux002

190 Schreeg, L. A., Mack, M. C., Turner, B. L. (2013). Nutrient-specific solubility patterns of leaf
191 litter across 41 lowland tropical woody species. *Ecology*, 94, 94-105. doi: 10.1890/11-
192 1958.1

193 Seelen, L. M. S., Flaim, G., Keuskamp, J., Teurlincx, S., Font, R. A., Tolunay, D., . . . Domis,
194 L. N. D. (2019). An affordable and reliable assessment of aquatic decomposition:

195 Tailoring the Tea Bag Index to surface waters. *Water Research*, 151, 31-43.
 196 doi:10.1016/j.watres.2018.11.081

197 Toth, Z., Hornung, E., & Baldi, A. (2018). Effects of set-aside management on certain elements
 198 of soil biota and early stage organic matter decomposition in a High Nature Value Area,
 199 Hungary. *Nature Conservation-Bulgaria*, 29, 1-26.
 200 doi:10.3897/natureconservation.29.24856

201 Toth, Z., Tancsics, A., Kriszt, B., Kroel-Dulay, G., Onodi, G., & Hornung, E. (2017). Extreme
 202 effects of drought on composition of the soil bacterial community and decomposition of
 203 plant tissue. *European Journal of Soil Science*, 68(4), 504-513. doi:10.1111/ejss.12429

204 Wang, L. F., Chen, Y. M., Zhou, Y., Xu, Z. F., Tan, B., You, C. M., . . . Liu, Y. (2021).
 205 Environmental conditions and litter nutrients are key determinants of soluble C, N, and
 206 P release during litter mixture decomposition. *Soil & Tillage Research*, 209.
 207 doi:10.1016/j.still.2020.104928

208 Xiong, S. J., & Nilsson, C. (1997). Dynamics of leaf litter accumulation and it effects on riparian
 209 vegetation: A review. *Botanical Review*, 63(3), 240-264. doi:10.1007/bf02857951

210

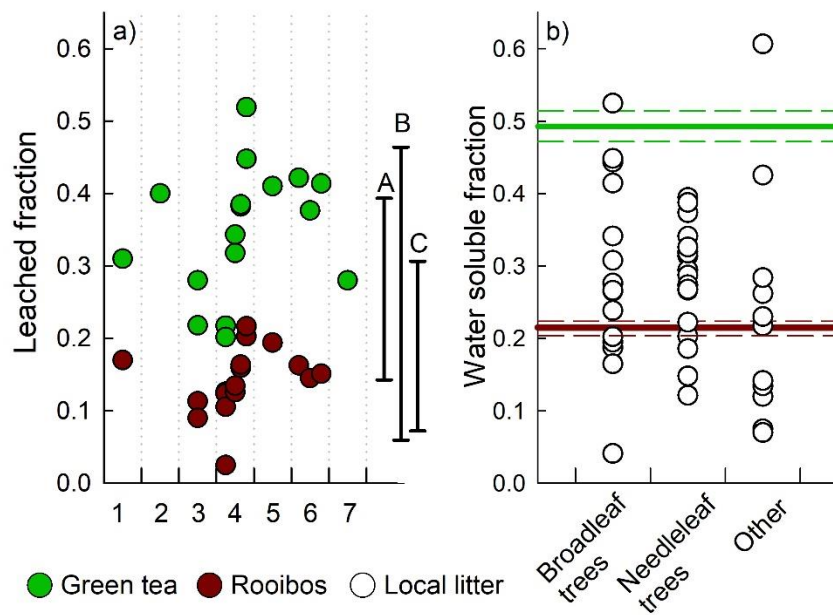


Figure 1: a) variation in leaching estimates of rooibos and green tea in literature sorted from short to long incubation durations. Grey shaded areas represent the ranges of leaching of local litter reported in A; Friesen et al. (2018), B; Jiang et al. (2016) and C; Xiong and Nilsson (1997). Study numbers on the x-axis are 1; Djukic et al. (2018), (3 min at 100°C), 2; Pouyat et al. (2017) (80 min; 60°C: only green tea), 3; Seelen et al. (2019), (3h; outdoor 9.5-14°C), 4; Lind et al. (2022), (3h; from left to right: outdoor measurements, 8, 19 and 60°C), 5; Blume-Werry et al. (2021), (12h; 25°C), 6; Mori et al. (2021) (24h; 3, 15 and 25°C), 7; Madaschi and Diaz-Villanueva (2021), (48h; room temperature: only green tea). b) Variation in water soluble fraction in tea and other plant material (Harmon, 2016) with the red and the green line representing the initial water-soluble fraction of rooibos and green tea respectively and their standard deviation (Keuskamp et al., 2013). The category 'other' includes graminoids, some lichens but no forbs.

Data Accessibility Statement: Data obtained from literature sources (Blume-Werry et al., 2021; Djukic et al., 2018; Friesen et al., 2018; Harmon, 2016; Jiang et al., 2016; Keuskamp et al., 2013; Lind et al., 2022; Madaschi and Diaz-Villanueva, 2021; Mori et al., 2021; Pouyat et al., 2017; Seelen et al., 2019; Xiong and Nilsson, 1997).

Competing Interests Statement: We declare no conflict of interest.

Author Contributions:

Judith M. Sarneel: Conceptualization (equal); investigation (equal); visualization (lead); writing – original draft (lead); writing – review and editing (lead). **Janna M.**

Barel: Conceptualization (equal); investigation (supporting); writing – review and editing (equal). **Sarah Duddigan:** Conceptualization (equal); writing – review and editing

(equal). **Joost A. Keuskamp:** Conceptualization (equal); writing – review and editing

(equal). **Ada Pastor Oliveras:** Conceptualization (equal); writing – review and editing

(equal). **Taru Sanden:** Conceptualization (supporting); writing – review and editing (equal).

Gesche Blume-Werry: Conceptualization (equal); investigation (lead); writing – review and editing (equal)

Acknowledgements: JMS acknowledges Formas for Funding (2022-02449).