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

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Abstract

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

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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 al. (2022), we believe that correcting for leaching in (terrestrial) litterbags studies such as the 26 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

34 Introduction

35 During litter decomposition, a fraction of the water-soluble components of the litter is quickly 36 dissolved (leached) into the water that is available in the environment. Besides leaching, litter 37 decomposition is driven by fragmentation, (UV)-bleaching and microbial activity. Many studies quantify litter decomposition by measuring mass-loss rates of incubated leaves, which 38 39 inherently integrate the biotic and abiotic processes that drive litter decomposition. In 2013, 40 the Tea Bag Index (TBI) was published, which is an easy method that uses tea bags as 41 equivalent to litter bags filled with local litter (Keuskamp et al., 2013). Because the plant 42 material used as litter in the TBI has a leaching product we are all familiar with, tea, it inspired 43 Lind et al. (2022) and others (Figure 1a) to explicitly address and quantify leaching. In addition, 44 frameworks like the Microbial Efficiency-Matrix Stabilization (Cotrufo et al., 2013) and 45 increased interest in fluxes of dissolved organic matter from soils (Cleveland et al., 2004) 46 further highlight the role of leaching during litter decomposition. Mechanistic studies such as 47 presented by Lind et al. (2022) contribute to an increased understanding of the factors that 48 drive leaching losses during litter decomposition. However, we disagree with the conclusion 49 Lind et al. (2022) draw from their studies; that there is a need to introduce a leaching correction 50 in the Tea Bag Index. We believe that correcting for leaching (especially in terrestrial TBI and 51 other mass-loss based studies) introduces more uncertainties than it solves. As a result, 52 correcting for leaching hampers the interpretation, decreases comparability across studies and 53 increases the complexity of the TBI that is designed to be a simple method.

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55 **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

62 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 63 64 definitions in order to minimize confusion in the scientific discussion. Moreover, there are two 65 common approaches to account for leaching in mass loss studies, which both may have their own implications. The first is a posteriori mathematical correction of the initial weight based on 66 67 a local measurement of the weight loss during a short period of time (Lind et al., 2022; Seelen 68 et al., 2019). Alternatively, litterbags are soaked before incubation to remove most of the water-69 soluble material (in TBI; Blume-Werry et al., 2021; Kotze & Setala, 2022; Toth et al., 2018; 70 Toth et al., 2017). In this comment, we will focus on the *a posteriori* correction.

71

72 Leaching in tea

73 The TBI consists of burying two types of tea bags as an easy alternative for litter bags filled 74 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 75 76 estimates the decay of the soluble and hydrolysable compounds in rooibos tea. Although we 77 do not claim that the tea used in TBI completely represents local litter material, the water-78 soluble fraction of tea (the total of leachable material) is well in range with other litter (Figure 79 1b; Harmon, 2016). We therefore disagree with the statement of Lind et al. (2022) that 'initial 80 leaching of water-soluble compounds may therefore be even higher in the tea bag 81 decomposition substrates than for intact leaves of traditional litterbag studies'. Moreover, 82 leaching measurements by Lind et al (2022) include extremes when compared to other 83 leaching measurements in tea. On average, leaching in rooibos and green tea is within the 84 ranges reported in the three review studies to our knowledge available on leaching (mass loss 85 of 14- 40%, 5.7 - 47.2% and 7-31%, respectively; Friesen et al., 2018; Jiang et al., 2016; Xiong 86 & Nilsson, 1997; Figure 1).

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88 **Reasons against a leaching correction**

Lind et al. (2022) advocate that correcting litter decomposition rates for leaching would improve 89 90 the TBI method (and implicitly other litterbag studies). The TBI method intends to obtain a 91 standardized, easy measurement of mass losses and introducing a leaching correction would 92 complicate both its practical use as well its' interpretation. Hence, such correction would throw 93 the baby out with the (leached) bathwater. Specifically, we believe that a leaching correction 94 would introduce a number of uncertainties: Firstly, leaching is a continuous process as both 95 starting products and products resulting from degradation can be leached when environmental 96 conditions allow (Wang et al., 2021). This implies that rain events, or (as Lind et al. (2022 97 show) temperature changes may cause additional leaching (Wang et al., 2021). Even when 98 the aim is to only correct for initial leaching of the fresh litter, the timeframe in which mass loss 99 is uniquely due to leaching will remain an educated guess. Moreover, initial leaching duration 100 may differ between ecosystems, between seasons within the same ecosystem, due to variation 101 in temperature and water availability and unpredictable precipitation events (Lind et al., 2022). 102 That this is uncertainty is felt by the researching community is reflected by the variable duration 103 of leaching measurements that are applied: For instance, leaching measurements of tea were 104 conducted from 3 minutes to 48 hours (Figure 1). Other than in terrestrial systems, leaching 105 as an initial event is possible to quantify in aquatic systems (Elwood et al., 1981; Gessner et 106 al., 1999; Seelen et al., 2019), but also in this systems, mass-loss studies frequently do not 107 use a leaching correction but integrate all processes that cause litter material to disintegrate 108 (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 excactly 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).

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126 Conclusion

127 Lind et al. (2022) convincingly show that the same factors (temperature and moisture) that 128 affect mineralization can also drive differences in leaching, and flag for higher appreciation of 129 this process in the TBI, mass-loss and litterbag studies. Yet, making a mathematical correction 130 of leaching part of the standardized TBI method is not feasible or desirable. It introduces more 131 uncertainties than it solves and undermines the purpose of the method: standardisation 132 between studies. The TBI was designed to be an easy and reproduceable way to study litter 133 mass loss, by both professional scientists and citizen scientists. TBI, like many other litter bag 134 studies, includes the environmental effects on fragmentation, leaching, bleaching and 135 mineralization. Tea bags could potentially help to disentangle the environmental variables that 136 drive leaching. Future litter decomposition and leaching studies will improve by careful 137 interpretation of solid experiments, being transparent about definitions used and explaining the 138 way in which leaching corrections were applied. Comparison across studies is further 139 enhanced by standardization of the methods used, and as outlined above, a correction for 140 leaching is not advised in TBI.

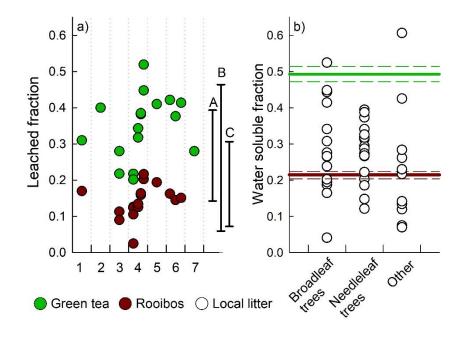
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212 Figure 1: a) variation in leaching estimates of rooibos and green tea in literature sorted from 213 short to long incubation durations. Grey shaded areas represent the ranges of leaching of local 214 litter reported in A; Friesen et al. (2018), B; Jiang et al. (2016) and C; Xiong and Nilsson (1997). 215 Study numbers on the x-axis are 1; Djukic et al. (2018), (3 min at 100°C), 2; Pouyat et al. (2017) 216 (80 min; 60°C: only green tea), 3; Seelen et al. (2019), (3h; outdoor 9.5-14°C), 4; Lind et al. 217 (2022), (3h; from left to right: outdoor measurements, 8, 19 and 60°C), 5; Blume-Werry et al. 218 (2021), (12h; 25°C), 6; Mori et al. (2021) (24h; 3, 15 and 25°C), 7; Madaschi and Diaz-219 Villanueva (2021), (48h; room temperature: only green tea). b) Variation in water soluble 220 fraction in tea and other plant material (Harmon, 2016) with the red and the green line 221 representing the initial water-soluble fraction of rooibos and green tea respectively and their 222 standard deviation (Keuskamp et al., 2013). The category 'other' includes graminoids, some 223 lichens but no forbs.

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- 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).
- 230
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- 234 Judith M. Sarneel: Conceptualization (equal); investigation (equal); visualization (lead);
- writing original draft (lead); writing review and editing (lead). Janna M.
- 236 Barel: Conceptualization (equal); investigation (supporting); writing review and editing
- 237 (equal). Sarah Duddigan: Conceptualization (equal); writing review and editing
- 238 (equal). Joost A. Keuskamp: Conceptualization (equal); writing review and editing
- 239 (equal). Ada Pastor Oliveras: Conceptualization (equal); writing review and editing
- 240 (equal). Taru Sanden: Conceptualization (supporting); writing review and editing (equal).
- 241 **Gesche Blume-Werry:** Conceptualization (equal); investigation (lead); writing review and
- 242 editing (equal)
- 243
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