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Towards Harmonizing Leaf Litter Decomposition Studies Using Standard Tea Bags—A Field Study and Model Application

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Abstract: Decomposition of plant litter is a key process for the transfer of carbon and nutrients in ecosystems. Carbon contained in the decaying biomass is released to the atmosphere as respired CO₂, and may contribute to global warming. Litterbag studies have been used to improve our knowledge of the drivers of litter decomposition, but they lack comparability because litter quality is plant species-specific. The use of commercial tea bags as a standard substrate was suggested in order to harmonize studies, where green tea and rooibos represent more labile and more recalcitrant C compounds as surrogates of local litter. Here we examine the potential of the use of standardized material for improving our understanding of litter decomposition across climate regions, and to further develop pertinent models. We measured the decomposition of incubated local and standard litters over two years along an elevation gradient in the Austrian Limestone Alps. The similar response to changes in temperature and precipitation of the pairs of local and standard litter—i.e., *Fagus sylvatica* and green tea, and *Pinus nigra* and rooibos tea, respectively—suggests the suitability of the standard litters for further examining the role of environmental drivers of decomposition. Harmonized data obtained from standardized litter experiments would provide a key prerequisite for further developing simulation models for the estimation of the C balance of ecosystem litter pools.

Keywords: carbon; climosequence; comparability; litterbags; litter decomposition; modeling; Yasso15; verification; validation

1. Introduction

Plant growth, senescence, and mortality result in a continuous supply of organic matter, such as foliage, which accumulates as plant litter on or in the soil to be decomposed by microorganisms. Decomposition of plant litter is a key process for the flow, recirculation, and storage of energy, carbon, and nutrients in ecosystems, and it is controlled by site conditions including temperature and precipitation, substrate availability, and decomposing organisms (as reviewed in [1]). Hence, the decomposition rates of litter can vary considerably across ecosystems. Decomposition of plant litter and soil organic matter represents one of the largest fluxes in the global terrestrial C cycle, contributing approximately 60 Pg C·year⁻¹ [2], and it can contribute significantly to annual CO₂ emissions from vegetated ecosystems such as forests, crop-, and grasslands. It is known that considerable uncertainty

remains on the decomposition dynamics of plant litter (e.g., [3,4]), and thus in the estimates of CO₂ emissions from this litter pool.

Litterbags have been used extensively to examine the importance of different drivers and how they affect the nutrient and mineral fluxes from the plant litter to the soil (e.g., [5–7]). Based on a meta-analysis of 66 litterbag experiments involving 818 plant species, Cornwell et al. [8] showed that within a climate region, plant species traits are important drivers of their litter decomposition. Although these studies have provided important insights into the site-specific litter decomposition dynamics, the comparability between studies and sites is hampered because they (generally) have used local litter (e.g., [8–12]). To identify common drivers of litter decomposition at regional or global scales, the bias which was introduced by using local litter and species-specific decomposition records needs to be removed.

The Decomposition Study in Europe (DECO; [13,14]) attempted to circumvent this limitation by including litter bags with similar “standardized” pine litter types from Sweden across a large number of European sites. However, such methods involving the production of standardized litterbags have clear limitations, for logistic and economic reasons. Keuskamp et al. [15] suggested the use of tea as a standard substrate with different decomposition rates to harmonize decomposition studies across sites and ecosystem types. Such a standardized approach involves several advantages, including that it is a simple, cost-, and time-efficient method using material that is globally available in the same quality, and even potentially engages in “citizen science” [16]. Moreover, a tea bag approach would enable high-resolution decomposition measurements in space and time, which would provide unique data for calibration and up-scaling to ecosystems and for modeling efforts enabling a more accurate quantification of litter decomposition processes. The method suggests the use of fast-decomposing green tea and slower decomposing rooibos tea. The application of two litter types with different decay rates allows the study and reproduction of hypotheses on the differences between more labile and more recalcitrant C compounds (e.g., [17]). The tea bag approach, however, will only provide information about the potential decomposition rates in a given ecosystem or site. In order to provide a link between the potential (i.e., based on standardized tea litter) and actual (i.e., based on local litter) decomposition rates, simultaneous incubation of native litter would be needed.

This study presents a first attempt to link the measured decomposition of tea and local litter. Here we compare litter decay rates between tea bags and local leaf litter along an elevation gradient in the Austrian Limestone Alps, ranging from deciduous and conifer-dominated forests to alpine grassland [12]. In addition to litter decomposition measurements, we simulated the decomposition of natural litter and teas with the litter and soil carbon model Yasso15 [18]. The model was selected because a previous model version (Yasso07) was shown to accurately reproduce tree foliage and fine root litter in an Alpine environment [19].

The main objective of this study is to explore the opportunities that the use of standardized material provides for studying decomposition processes within and across ecosystem types. The study will allow the identification of the potential of standardized measurements to improve the knowledge of litter decomposition and to develop and verify models. An implementation of this approach in different ecosystems could then be used to identify regionally and globally important drivers of decomposition, which is not possible by using local litter. In particular, the study pursues several aims: (1) Examining the suitability of standard substrate (tea) for harmonizing litter decomposition studies; (2) exploring the utility of green tea and rooibos as surrogates of faster and slower decomposing local litter types; and (3) simulating observed decomposition of native and standard litter with Yasso15 to evaluate the opportunities of the tea bag approach for further development of pertinent simulation models. Consistent with the laboratory analysis of green tea and rooibos by Keuskamp et al. [15], we expected to find that green tea decomposes faster than rooibos. Since standard and local litter differed in their chemical composition [15,19,20], differences in their decomposition rates were expected. Nevertheless, we anticipated that the temporal decomposition pattern of the standard material follows

that of the local litter. For the simulations, we expected that the model would reproduce the observed differences in the decomposition of the two standard litter types.

2. Materials and Methods

2.1. Study Area

The study area was located in the Northern Limestone Alps of Austria on a south–southwest slope of the Hochschwab Mountain. Along an elevation gradient from montane to the subalpine and alpine climate zone, three study sites were established, ranging from deciduous (900 m above sea level (asl)) and coniferous forest (1300 m asl) to alpine grassland (1900 m asl). This elevation gradient represents a climosequence, with long-term mean annual temperature decreasing from 6.2 to 2.1 °C, and mean annual precipitation increasing from 1178 to 1725 mm (source: [21]; Figure 1). The soils are Leptic Histosols [22] formed on calcareous parent material, and are characterized by high organic carbon contents and slightly acidic topsoil (Figure 1).

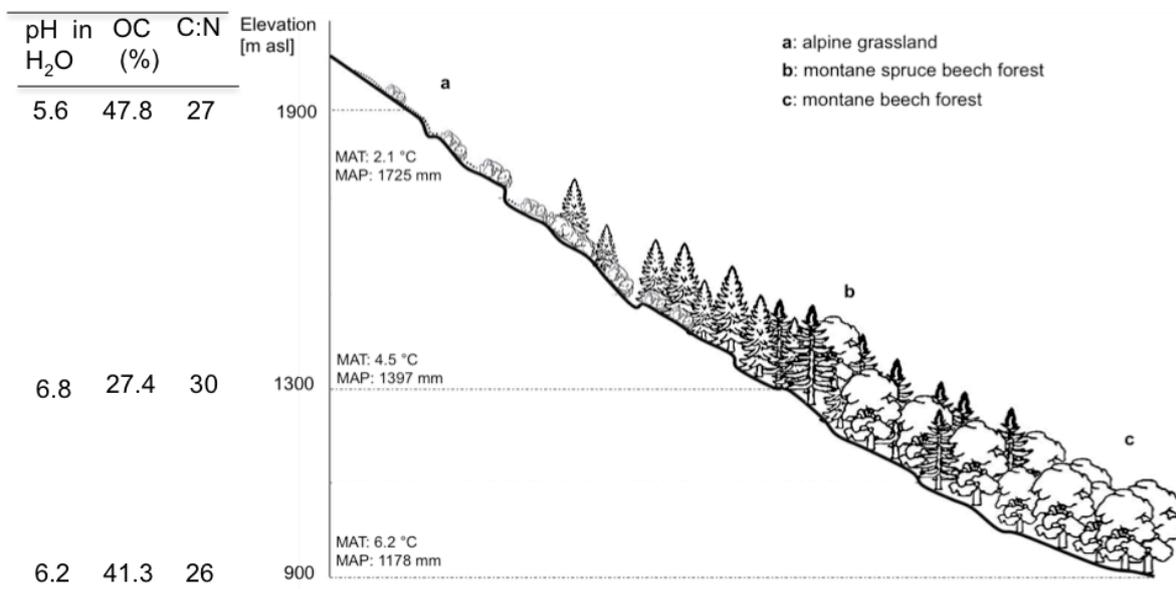


Figure 1. Studied climosequence with the basic site and soil (0–5 cm depth) characteristics. MAT, long-term mean annual temperature; MAP, long-term mean annual precipitation; m asl, meters above sea level; OC, organic carbon.

2.2. Litter Decomposition Measurements

2.2.1. Local Litter

Intact, newly shed leaves of *Fagus sylvatica* and needles of *Pinus nigra* were collected from the forest floor and dried at 50 °C until the weight remained constant. The litterbags were made of polyethylene nets (10 × 10 cm; 1 mm mesh size) and filled with 1.5 g of *F. sylvatica* leaves and 3 g of *P. nigra* needles, respectively. In June 2007, on each of the three study sites, 20 litter bags (five replicates per litter type and two sampling campaigns over two years) were buried into the soil at 5 cm depth. Litterbags were collected after one (June 2008) and two (June 2009) years of incubation. The cleaned litter samples were dried at 105 °C until the weight remained constant and were ground for the further analyses. For a more detailed description of the study see Djukic et al. [23].

2.2.2. Tea Bag Litter

Lipton (Katowice Factory, Katowice, Poland) green tea (EAN No.: 87 22700 05552 5) and Lipton rooibos tea (EAN No.: 87 22700 18843 8) bags are made out of synthetic material with a mesh size of 0.25 mm and are filled with 2 g of green and rooibos tea, respectively [15]. Before the incubation, the tea was dried at 70 °C until the weight remained constant. Similar to the study with local litter, at each study site 20 tea bags (five replicates per type and year) were buried in the soil at 5 cm depth and incubated over a two year period starting in June 2011. Tea bags were collected in June 2012 and June 2013. The cleaned litter samples were dried at 70 °C until the weight remained constant and litter mass loss was calculated.

2.3. Litter Decomposition Simulations

The decomposition of the natural and standard litters was simulated using the user-interface version of the litter decomposition and soil carbon model Yasso15 [18,24]. Yasso15 has the same structure and is based on the same ideas as an earlier model version, Yasso07 [24–27]. However, Yasso15 is based on an extended dataset, and consequently, there are differences in the parameter values.

Yasso15 divides litter into four compound groups that decompose at their unique rates. The groups are compounds (1) hydrolysable in acid (denoted with A); (2) soluble in water (W); (3) in a non-polar solvent, i.e., ethanol or dichloromethane (E); and (4) neither soluble nor hydrolysable (N). In addition, there is a fifth more recalcitrant compound group (H) formed of the decomposition products of the A, W, E, and N groups. Decomposition results in carbon fluxes between the groups and into the atmosphere. The decomposition rates are controlled by air temperature and precipitation.

In the simulations, the chemical compositions of the standard litter were based on measurements [15], and those of natural litters on mean values of measurements of different *Fagus* and *Pinus* species ([20]; Table 1). To account for the uncertainty in the chemical composition, a standard deviation equal to 10% was applied to each of the compound groups in the simulations. The mean annual temperature and temperature amplitude were calculated from hourly data measured at the study sites in 2007 and 2008 (Table 2). These values were used in all of the simulations because temperature data was missing for several months during the tea bag experiment in 2011–2013. The data loggers did not provide precipitation data for winter months at the study sites, and, for this reason, we used the average annual precipitation of the time period 1961–1990 from nearby weather stations (source: [21]). The user-interface of the Yasso15 model allows the user to take into account the mesh size of litter bags using a leaching parameter [18]. In this study, we used a leaching parameter value equal to -0.154872 for the natural litters (litter bag mesh size 1 mm \times 1 mm), and a value equal to -0.000404 for the teas (mesh size 0.2 mm \times 0.3 mm; Yasso15 manual available at [28]).

Table 1. The mean chemical composition of natural litter and tea used in the Yasso15 simulations based on Gholz et al [20] and Keuskamp et al. [15], respectively. The values present fractions of compounds hydrolysable in acid (A), compounds soluble in water (W), in a non-polar solvent, ethanol or dichloromethane (E), and compounds neither soluble nor hydrolysable (N).

	A	W	E	N
Green Tea	0.283	0.493	0.066	0.156
Rooibos Tea	0.289	0.215	0.049	0.444
<i>Fagus</i> sp.	0.498	0.165	0.074	0.264
<i>Pinus</i> sp.	0.421	0.202	0.172	0.205

Table 2. Annual climate used in the Yasso15 simulations based on measured data from 2007 to 2008.

Elevation	900 m		1300 m		1900 m	
Year	2007	2008	2007	2008	2007	2008
Mean annual temperature (°C)	7.2	7.0	5.7	5.4	3.6	4.9
Temperature amplitude (°C)	7.6	6.9	5.9	5.7	6.9	5.8
Annual precipitation (mm)	967	967	1397	1397	1725	1725

The temperature amplitude is a half of the difference between the mean temperatures of the warmest and the coldest month of the year.

2.4. Data Analysis

Statistical analyses to examine the effect of elevation and litter type were performed with SPSS for Windows. One-way ANOVA with Tukey post hoc test ($p < 0.05$) was used for identification of statistically different groups. The dependency of observed and simulated litter decomposition on temperature and precipitation was examined using linear regression and correlation (Pearson's product-moment correlation) analysis in R [29].

3. Results

3.1. Observed Decomposition of Standard and Local Litter

The decomposition of all four litter types showed the same trend with elevation. The decomposition rate decreased with elevation, and hence, more mass remained at higher elevation (Figure 2). The mass loss of local litter was lower in the second year than in the first year. On average over all sites, *F. sylvatica* and *P. nigra* litters lost 36% and 32%, respectively, of their initial mass during the first year. During the second year, the mass loss was 24% and 22%, respectively. The average mass loss of the standardized material during the first year was 34% for green tea and 7% for rooibos tea. During the second year, four of the five replicates of green tea gained slightly in mass, while rooibos tea mass decreased by 11% on average.

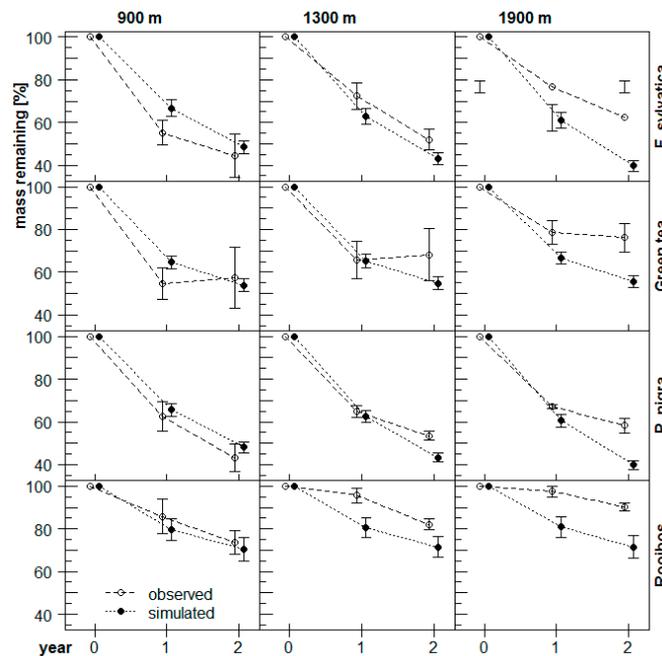


Figure 2. Mean and 95% confidence intervals of observed ($n = 5$) and simulated ($n = 1000$) mass loss after one and two years for *Fagus sylvatica*, *Pinus nigra*, green tea, and rooibos tea at three elevations. Note that the symbols for observed and simulated data are slightly offset for better distinction.

The effect of a change in elevation was strongest for green tea and *F. sylvatica* litter, compared to rooibos tea and *P. nigra* litter. First year mass remaining of the green tea was in similar order as the mass remaining of *F. sylvatica*, while during the second year, significant differences were observed at the two high-elevation sites (Figure 2). At the lower-elevation forested site (900 m asl), the mass remaining was not significantly different from the native litter during the second year of decomposition. On the other hand, the mass remaining of the rooibos tea was significantly different from the decomposition of the *P. nigra* litter during the entire incubation time (Table 3).

Table 3. Multiple comparison (*p*-values) between the mass loss of the native litter (*Fagus sylvatica* and *Pinus nigra*) and the standard litter (Green tea and Rooibos tea) during the two years of decomposition along the studied gradient (ANOVA, Tukey post hoc test, *p* < 0.05, *n* = 5).

Elevation (m asl)	Green Tea against <i>Fagus sylvatica</i> Litter		Rooibos Tea against <i>Pinus nigra</i> Litter	
	Year 1	Year 2	Year 1	Year 2
1900	0.724	0.000	0.000	0.000
1300	0.165	0.001	0.000	0.000
900	0.999	0.086	0.000	0.000

Figure 3 separates the elevation change into temperature and precipitation effects. For observed decomposition, the pairs of local and standard litter (i.e., *F. sylvatica* and green tea, and *P. nigra* and rooibos tea) respectively showed the same response: an increase in mean annual temperature leads to a higher decomposition rate (i.e., less mass remained at higher temperature; Pearson’s *r* < −0.9, *p* < 0.1 in the case of local, and <0.05 in the case of standard litter), an increase in mean annual precipitation leads to a lower decomposition rate (i.e., more mass remained at higher precipitation; Pearson’s *r* > +0.9, *p* < 0.1 in the case of local, and <0.05 in the case of standard litter).

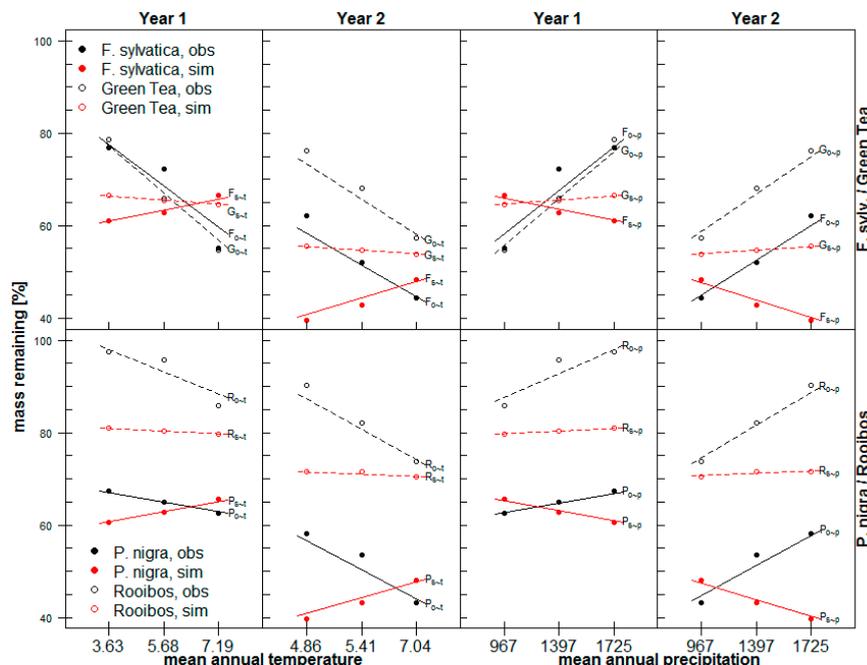


Figure 3. Temperature (mean annual; °C); t)- and precipitation (mean annual; mm); p)-dependence of mean observed (o) and simulated (s) mass remaining after one and two years for *Fagus sylvatica* (F), *Pinus nigra* (P), green tea (G), and rooibos tea (R). Solid (local litter) and dashed (standard litter) lines show the result of linear regressions between climate variables and remaining mass: observed *Fagus* against temperature and precipitation (F_{o-t}, F_{o-p}); simulated *Fagus* against temperature and precipitation (F_{s-t} (*p* < 0.05), F_{s-p} (*p* < 0.05)); green tea (G_{o-t}, G_{o-p} (*p* < 0.01); G_{s-t}, G_{s-p} (*p* < 0.05)); *Pinus* (P_{o-t}, P_{o-p} (*p* < 0.05); P_{s-t} (*p* < 0.05), P_{s-p} (*p* < 0.01)); rooibos tea (R_{o-t}, R_{o-p}; R_{s-t}, R_{s-p}); *p*-values in squared brackets give the significance level. Note that the same mean annual precipitation was applied to both years, which was due to technical problems with the rain gauge during one year.

3.2. Simulated Decomposition of Standard and Local Litter

Yasso15 reproduced the observations more accurately at lower elevations than at the highest elevation (Figure 2). The model estimates for the sites at 900 m and 1300 m were within the range of observed mass remaining in natural *F. sylvatica* and *P. nigra* litter after one and two years of incubation (Figure 2). The decomposition rate of native litter emerging from the model simulations increased with elevation, resulting in an overestimation of the mass loss rate at the 1900 m site.

Similarly to measurements, Yasso15 estimated faster mass loss for green tea than for rooibos tea. The model also captured the observed temporal decomposition dynamics: Green tea loses mass quickly at first, before the decomposition rate slows down; whereas for rooibos tea, mass loss rate is more stable over time. The observed trend of a reduction in decomposition rates with elevation was reproduced by the model, albeit more weakly (Figure 2). Some measurements indicate no mass loss from green tea bags. These measurements could not be reproduced with the model.

Relative mass loss was lower in the second year. During the first year, the average mass loss over all three elevation levels was 35% for green tea, 20% for rooibos tea, 37% for *F. sylvatica*, and 37% for *P. nigra*. The corresponding values were lower during the second year, with 16% for green tea, 11% for rooibos tea, 31% for *F. sylvatic*, and 31% for *P. nigra*.

The simulated decomposition rates of the native litters increased with an increase in elevation (i.e., less mass remained at higher elevation), whereas these rates did not vary according to elevation in the teas (Figure 2). In the cases of the native litters, the effect of increasing precipitation on the decomposition rate more than compensated for the effect of decreasing temperature. On the other hand, in the cases of the teas, the effect of the increasing precipitation was just enough to compensate for the effect of the decreasing temperature. Thus, these simulated trends in the decomposition rates were different than the trends in the measurements (Figure 3).

4. Discussion

4.1. Observed Decomposition of Standard and Local Litter

Our results from two years of observed and simulated decomposition suggest that local litter and green tea decay follows a negative exponential pattern, with the slowest decomposition observed at the high-elevation grassland site (Figure 2). Decomposition rates differed between local and standard litter, as was expected. For a more extensive application of a tea bag approach, it was promising to find the similar response to changes in temperature and precipitation of the pairs of local and standard litter; i.e., *F. sylvatica* and green tea, and *P. nigra* and rooibos tea, respectively.

The observed pattern of decomposition was consistent with general expectations. In the study area, Djukic et al. [23] showed that during the decomposition of maize straw, environmental and site variables were the most significant drivers during the first year of the decomposition, while in the second year, the effect of substrate availability and complexity increased [30]—and hence also the role of microorganisms. Indeed, the broad scale processes such as metabolism of labile C compounds is performed by many groups of microorganisms, while the decomposition of the more stable substrate is a narrow process and is conducted by only a subset of microbes [31]. Moreover, the significant decomposition differences between rooibos tea and *P. nigra* (Table 3) are suggestive of the existence of two distinct substrate types. While the rooibos tea constitutes a mixture of mainly needle-like litter and stem tissue, with a C:N ratio of 42.87 (± 1.84 SD) [15], the C:N ratio of the *P. nigra* litter is 87 (± 7.8 SD) (based on [12]). However, contrary to the expectation that mass loss decreases with an increase in the C:N ratio, *P. nigra* mass loss was higher than rooibos mass loss in this study. On the other hand, the results for *F. sylvatica* (C:N ratio of ca. 60) and *P. nigra* (ca. 87), as well as for green tea (ca. 12) and rooibos (ca. 43), were consistent with expectations and with data from a litterbag study in Switzerland that showed that higher C:N ratios result in slower decomposition [10]. The comparatively high proportion of non-soluble compounds in rooibos litter (Table 1) may be one explanation for the unexpected pattern, and should be examined in further studies of this kind.

The observed increase in mass during the second year of green tea decomposition was likely due to colonization by fungi, which was also observed in similar studies [32]. Additionally, an increase in ash content may be responsible for the observed increase in mass, as suggested by Berg and McClaugherty [33]. Since ash content was not analyzed neither in the native nor in the tea litters, this hypothesis could not be examined.

4.2. Simulated Decomposition of Standard and Local Litter

The simulated patterns of decomposition over the two year period (Figure 2) were consequences of the chemical composition of the litter types. Green tea contained a high fraction of the easily decomposable W compound (Table 1). For this reason, it decomposed quickly at first; however, the decomposition rate slowed down after the majority of the original W fraction was used up. Rooibos tea, on the other hand, had a high amount of the more resistant N fraction (Table 1), and consequently, decomposed slowly over the entire two-year study period. The native litters decomposed more evenly than the teas because they contained a high fraction of the A compound (Table 1), which has a medium decomposition rate in the Yasso15 model.

The variability in the simulated decomposition patterns between the study sites resulted from combined effects of temperature and precipitation. Temperature decreased with increasing elevation, and precipitation increased with elevation (Table 2, Figure 1). In the Yasso15 model, decomposition rates increase towards warmer and high-precipitation conditions. In this study, the increase in precipitation from 967 to 1725 mm along the elevation gradient compensated for the decrease in annual mean temperature from 7.0 or 7.2 to 3.6 or 4.9 °C. It is noteworthy that the details of these combined temperature and precipitation effects varied among the litter types. This is because these effects are compound-group-specific in Yasso15, and the litter types had different concentrations of these groups (Table 1). The A, W, and E groups are the most sensitive, the N group is moderately sensitive, and the H group is the least sensitive.

4.3. Observed Versus Simulated Decomposition

The observed decomposition patterns of local and standard litter, such as the changes in the rate of decomposition during the first and the second year, were reproduced. The model also reproduced the observed differences between the four litter types. The agreement between observed and simulated decomposition was best at the lowest elevation site (Figure 2). Especially at the high elevation site at 1900 m, Yasso15 overestimated the rate of decomposition for all four litter types. This may be due to the low sensitivity of the model to the decrease in temperature or a too-high sensitivity to the increase in precipitation, as demonstrated in Figure 3. These inconsistencies may also be responsible for the opposing trend that more mass of *F. sylvatica* and *P. nigra* litter remained at higher temperature as compared to observation. While variability among replicated samples in the field study ($n = 5$) decreased with elevation, variability was similar at all sites in the simulated ($n = 1000$) data (Figure 2). This further suggests that the model may have a lower sensitivity to changes in climate, although the number of replicated simulations was high and may have contributed to the fact that variability in the simulations was low.

Considering that the model was built using a large global dataset [26], deviations from site-specific observations were expected, although the good agreement at the lower elevation site indicates the ability of the model to reproduce the observed differences in the four litter types and the effect of temperature and precipitation on decomposition. The study thus suggests the suitability of the Yasso15 model for application in a larger study with standardized litter to identify the role of abiotic drivers. Although the results show that the model moderately overestimated observed decomposition of standardized litter, this is likely not a model inconsistency. The differences in temperature between the three study sites, especially, are not very large, and the model may not be sensitive enough to pick this up, as other factors (e.g., chemical composition) may dominate. The low sensitivity of the model to factors which change little at the local scale is not surprising, since the model was developed for

general application ([26]) and an approach that may not be able to reproduce all detailed local effects ([34,35]). Furthermore, Didion et al. [19] demonstrated the accuracy of a previous version of the model at larger environmental gradients than were present in this study.

Yasso15 was used in this study as it is, without any calibration to the local conditions. The Yasso15 approach allows the addition of new measurements to the database and recalibration of the model using the extended database. This approach makes it possible to improve the suitability of Yasso15 in these studies. Combining measurements and modeling is very useful, because it helps us to understand the measured results on one hand, and on the other hand, improve the models, which provides an effective means of generalizing the measurements.

4.4. Potentials of the Use of Standardized Litter

The good agreement in the decomposition patterns between green tea and *F. sylvatica* on one hand, and rooibos tea and *P. nigra* on the other support the assumption that the two standardized litter types (green tea and rooibos tea) are suitable for further examination of the role of environmental drivers for litter decomposition; one objective of this study was to identify the suitability of this approach. Since the implementation of studies using tea bags is straightforward, an extensive application would contribute to further development of our understanding of litter decomposition. Indeed, the global International Long Term Ecological Research (ILTER) network [36] has initiated a research campaign “Global litter decomposition study”, which includes a variety of ecosystem types on different continents. Studying the complex ecosystem interactions requires long-term studies in order to provide temporal information at annual or finer resolution on the influence of climatic/site conditions, substrate quantity and quality on litter decomposition (i.e., contribution of litter decomposition on C-losses). Such studies may also provide more evidence on the slow-phase of decomposition process (i.e., C-storage), which would improve the existing knowledge of processes and drivers of decomposition, including the development of conceptual or analytical models describing litter decomposition (e.g., [5,37–39]). To this end, the duration of tea bag experiments should be extended in time to ensure that different phases of decomposition can be observed as documented by, for example, Berg et al. [40]. In addition, only by gathering data over extended periods of time can model predictions be verified.

The use of commercially available tea bags will reduce any variation related to litter bag preparation (e.g., mesh size), as well as workload, and will thus provide a solid basis for high-resolution measurements. Studies following a common protocol and using standardized litter will enable the comparison of decomposition rates across sites and ecosystems and avoid common inconsistencies due to, for example, different mesh sizes [41] or placement of the litterbags [4]. Additionally, harmonized data obtained from a large scale study would provide a key prerequisite for the further development of simulation models. Existing models to simulate C cycling in litter and soil (e.g., C-Tool [42], Romul [43], RothC [44], or Yasso [45]), typically differ in the representation of decay processes as, for example, in the extent of representing litter quality. The models were also developed for use in different ecosystems and at different scales [34]. Based on concurrent application of different models to simulate the decomposition of standardized litter, it would be possible to identify the significance of the underlying model assumptions and of knowledge gaps. Such model comparisons could also be used to strengthen the confidence in model estimates. A robust comparison between models is still difficult because suitable data are lacking to examine the role of process-detail for model predictions, even for the same land cover type (the study on models for reporting CO₂ emissions from forest litter and soil by Didion et al. [34]).

Since the approach using tea bags is a fairly recent development, additional studies under controlled conditions may be valuable to identify possible limitations. Due to particular characteristics of tea leaves (tea leaves are naturally flavored) [15], observed decomposition may be biased due to, for example, invasion by fungi or attracting rodents. Analyses of tea bag composition under controlled conditions would be required to identify potential biases. Controlled studies may also help to identify

the role of different abiotic drivers in more detail, since litter quality is constant and drivers (e.g., temperature) can also be kept constant.

5. Conclusions

This study provided a first attempt to further develop and apply a recent method, and to investigate its potential for improving our understanding of the decay process of plant litter across regions and vegetation types. The results presented here give a first evidence for the suitability of the approach using rooibos and green tea litters as standard litter to study environmental drivers of litter decomposition. Based on the incubation experiments with the established method using local litter and with the tea litters, the study showed that the dynamics of tea litter decomposition are consistent with those of the native litters. The findings of this study are important for the commencing large-scale application of the method, as needs for further improvements are identified and, particularly, confidence in the approach is created. The concurrent application of a simulation model to reproduce the observed decay of the local and standard litters showed that data from standardized litter experiments could provide a key prerequisite for the further development of pertinent models.

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Author Contributions: I.D. developed the concept of comparing local and tea bag litter and conceived the Global litter decomposition study together with M.B. M.D. together with I.D. conceived the study on comparing local and tea bag litter and observed and simulated decomposition. A.R., M.F., and J.L. carried out the simulations with Yasso15. M.D. wrote the paper with contributions from all authors. All authors contributed to the analysis.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Gavazov, K.S. Dynamics of alpine plant litter decomposition in a changing climate. *Plant Soil* **2010**, *337*, 19–32. [[CrossRef](#)]
2. Houghton, R.A. Balancing the global carbon budget. *Annu. Rev. Earth Planet. Sci.* **2007**, *35*, 313–347. [[CrossRef](#)]
3. Freschet, G.T.; Cornwell, W.K.; Wardle, D.A.; Elumeeva, T.G.; Liu, W.; Jackson, B.G.; Onipchenko, V.G.; Soudzilovskaia, N.A.; Tao, J.; Cornelissen, J.H.C. Linking litter decomposition of above- and below-ground organs to plant-soil feedbacks worldwide. *J. Ecol.* **2013**, *101*, 943–952. [[CrossRef](#)]
4. Bradford, M.A.; Berg, B.; Maynard, D.S.; Wieder, W.R.; Wood, S.A. Understanding the dominant controls on litter decomposition. *J. Ecol.* **2016**, *104*, 229–238. [[CrossRef](#)]
5. Berg, B. Decomposition patterns for foliar litter—A theory for influencing factors. *Soil Biol. Biochem.* **2014**, *78*, 222–232. [[CrossRef](#)]
6. Coûteaux, M.-M.; Bottner, P.; Berg, B. Litter decomposition, climate and litter quality. *Trends Ecol. Evol.* **1995**, *10*, 63–66. [[CrossRef](#)]
7. Hararuk, O.; Luo, Y. Improvement of global litter turnover rate predictions using a Bayesian MCMC approach. *Ecosphere* **2014**, *5*. [[CrossRef](#)]
8. Cornwell, W.K.; Cornelissen, J.H.C.; Amatangelo, K.; Dorrepaal, E.; Eviner, V.T.; Godoy, O.; Hobbie, S.E.; Hoorens, B.; Kurokawa, H.; Pérez-Harguindeguy, N.; et al. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecol. Lett.* **2008**, *11*, 1065–1071. [[CrossRef](#)] [[PubMed](#)]
9. Berger, T.W.; Duboc, O.; Djukic, I.; Tatzber, M.; Gerzabek, M.H.; Zehetner, F. Decomposition of beech (*Fagus sylvatica*) and pine (*Pinus nigra*) litter along an Alpine elevation gradient: Decay and nutrient release. *Geoderma* **2015**, *251–252*. [[CrossRef](#)] [[PubMed](#)]
10. Heim, A.; Frey, B. Early stage litter decomposition rates for Swiss forests. *Biogeochemistry* **2004**, *70*, 299–313. [[CrossRef](#)]

11. Lorenz, K.; Preston, C.; Krumrei, S.; Feger, K.-H. Decomposition of needle/leaf litter from Scots pine, black cherry, common oak and European beech at a conurbation forest site. *Eur. J. For. Res.* **2004**, *123*, 177–188. [[CrossRef](#)]
12. Duboc, O.; Zehetner, F.; Djukic, I.; Tatzber, M.; Berger, T.W.; Gerzabek, M.H. Decomposition of European beech and Black pine foliar litter along an Alpine elevation gradient: Mass loss and molecular characteristics. *Geoderma* **2012**, *189*, 522–531. [[CrossRef](#)]
13. Berg, B.; Staaf, H. Decomposition rate and chemical changes of scots pine needle litter. II. Influence of chemical composition. *Ecol. Bull.* **1980**, *32*, 373–390.
14. Jansson, P.-E.; Reurslag, A. Climatic influence on litter decomposition: Methods and some Results of a NW- European transect. In *Responses of Forest Ecosystems to Environmental Changes*; Teller, A., Mathy, P., Jeffers, J.N.R., Eds.; Springer: Dordrecht, The Netherlands, 1992; pp. 351–358.
15. Keuskamp, J.A.; Dingemans, B.J.J.; Lehtinen, T.; Sarneel, J.M.; Hefting, M.M. Tea bag index: A novel approach to collect uniform decomposition data across ecosystems. *Methods Ecol. Evol.* **2013**, *4*, 1070–1075. [[CrossRef](#)]
16. Teabag Index. Available online: <http://www.decolab.org/tbi/> (accessed on 26 July 2016).
17. Cotrufo, M.F.; Wallenstein, M.D.; Boot, C.M.; Deneff, K.; Paul, E. 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? *Glob. Chang. Biol.* **2013**, *19*, 988–995. [[CrossRef](#)] [[PubMed](#)]
18. Järvenpää, M.; (Tampere University of Technology, Department of Mathematics, Tampere, Finland); Repo, A.; (Finnish Environment Institute, Ecosystem Processes, Helsinki, Finland); Liski, J.; (Finnish Environment Institute, Ecosystem Processes, Helsinki, Finland); Kaasalainen, M.; (Tampere University of Technology, Department of Mathematics, Tampere, Finland). Unpublished work. 2016.
19. Didion, M.; Frey, B.; Rogiers, N.; Thürig, E. Validating tree litter decomposition in the Yasso07 carbon model. *Ecol. Model.* **2014**, *291*, 58–68. [[CrossRef](#)]
20. Gholz, H.L.; Wedin, D.A.; Smitherman, S.M.; Harmon, M.E.; Parton, W.J. Long-term dynamics of pine and hardwood litter in contrasting environments: Toward a global model of decomposition. *Glob. Chang. Biol.* **2000**, *6*, 751–765. [[CrossRef](#)]
21. Austrian Federal Ministry of Finance. Available online: <http://www.bundesfinanzministerium.de/Web/EN/Home/home.html> (accessed on 26 July 2016).
22. International Union of Soil Sciences (IUSS) Working Group World Reference Base for Soil Resources (WRB). *World Reference Base for Soil Resources 2006*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2006; p. 128.
23. Djukic, I.; Zehetner, F.; Watzinger, A.; Horacek, M.; Gerzabek, M.H. In situ carbon turnover dynamics and the role of soil microorganisms therein: A climate warming study in an Alpine ecosystem. *FEMS Microbiol. Ecol.* **2013**, *83*, 112–124. [[CrossRef](#)] [[PubMed](#)]
24. Tuomi, M.; Rasinmäki, J.; Repo, A.; Vanhala, P.; Liski, J. Soil carbon model Yasso07 graphical user interface. *Environ. Model. Softw.* **2011**, *26*, 1358–1362. [[CrossRef](#)]
25. Tuomi, M.; Laiho, R.; Repo, A.; Liski, J. Wood decomposition model for boreal forests. *Ecol. Model.* **2011**, *222*, 709–718. [[CrossRef](#)]
26. Tuomi, M.; Thum, T.; Järvinen, H.; Fronzek, S.; Berg, B.; Harmon, M.; Trofymow, J.A.; Sevanto, S.; Liski, J. Leaf litter decomposition—Estimates of global variability based on Yasso07 model. *Ecol. Model.* **2009**, *220*, 3362–3371. [[CrossRef](#)]
27. Tuomi, M.; Vanhala, P.; Karhu, K.; Fritze, H.; Liski, J. Heterotrophic soil respiration—Comparison of different models describing its temperature dependence. *Ecol. Model.* **2008**, *211*, 182–190. [[CrossRef](#)]
28. Soil carbon model—Yasso. Available online: <http://www.syke.fi/projects/yasso> (accessed on 26 July 2016).
29. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2015.
30. Duboc, O.; Dignac, M.-F.; Djukic, I.; Zehetner, F.; Gerzabek, M.H.; Rumpel, C. Lignin decomposition along an Alpine elevation gradient in relation to physicochemical and soil microbial parameters. *Glob. Chang. Biol.* **2014**, *20*, 2272–2285. [[CrossRef](#)] [[PubMed](#)]
31. McGuire, K.L.; Treseder, K.K. Microbial communities and their relevance for ecosystem models: Decomposition as a case study. *Soil Biol. Biochem.* **2010**, *42*, 529–535. [[CrossRef](#)]
32. Frey, B.; Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Forest Soils and Biogeochemistry, CH-8903, Birmensdorf, Switzerland. Personal communication. 16 March 2016.

33. Berg, B.; McClaugherty, C. *Plant Litter. Decomposition, Humus Formation, Carbon Sequestration*; Springer-Verlag: Berlin, Heidelberg, 2008.
34. Didion, M.; Blujdea, V.; Grassi, G.; Hernández, L.; Jandl, R.; Kriiska, K.; Lehtonen, A.; Saint-André, L. Models for reporting forest litter and soil C pools in national greenhouse gas inventories: Methodological considerations and requirements. *Carbon Manag.* **2016**, 1–14. [[CrossRef](#)]
35. Evans, M.R. Modelling ecological systems in a changing world. *Phil. Trans. R. Soc. B* **2012**, 367, 181–190. [[CrossRef](#)] [[PubMed](#)]
36. International Long Term Ecological Research. Available online: <http://www.ilternet.edu> (accessed on 26 July 2016).
37. Adair, E.C.; Parton, W.J.; del Grosso, S.J.; Silver, W.L.; Harmon, M.E.; Hall, S.A.; Burke, I.C.; Hart, S.C. Simple three-pool model accurately describes patterns of long-term litter decomposition in diverse climates. *Glob. Chang. Biol.* **2008**, 14, 2636–2660. [[CrossRef](#)]
38. Manzoni, S.; Piñeiro, G.; Jackson, R.B.; Jobbágy, E.G.; Kim, J.H.; Porporato, A. Analytical models of soil and litter decomposition: Solutions for mass loss and time-dependent decay rates. *Soil Biol. Biochem.* **2012**, 50, 66–76. [[CrossRef](#)]
39. Harmon, M.E.; Silver, W.L.; Fasth, B.; Chen, H.U.A.; Burke, I.C.; Parton, W.J.; Hart, S.C.; Currie, W.S. Long-term patterns of mass loss during the decomposition of leaf and fine root litter: An intersite comparison. *Glob. Chang. Biol.* **2009**, 15, 1320–1338. [[CrossRef](#)]
40. Berg, B.; Kjønaas, O.J.; Johansson, M.B.; Erhagen, B.; Åkerblom, S. Late stage pine litter decomposition: Relationship to litter N, Mn, and acid unhydrolyzable residue (AUR) concentrations and climatic factors. *For. Ecol. Manag.* **2015**, 358, 41–47. [[CrossRef](#)]
41. Bradford, M.A.; Tordoff, G.M.; Eggers, T.; Jones, T.H.; Newington, J.E. Microbiota, fauna, and mesh size interactions in litter decomposition. *Oikos* **2002**, 99, 317–323. [[CrossRef](#)]
42. Petersen, B.M.; Olesen, J.E.; Heidmann, T. A flexible tool for simulation of soil carbon turnover. *Ecol. Model.* **2002**, 151, 1–14. [[CrossRef](#)]
43. Chertov, O.G.; Komarov, A.S.; Nadporozhskaya, M.; Bykhovets, S.S.; Zudin, S.L. ROMUL—A model of forest soil organic matter dynamics as a substantial tool for forest ecosystem modeling. *Ecol. Model.* **2001**, 138, 289–308. [[CrossRef](#)]
44. Coleman, K.; Jenkinson, D.S. RothC-26.3—A model for the turnover of carbon in soil. In *Evaluation of Soil Organic Matter Models Using Existing Long-Term Datasets*; Powlson, D.S., Smith, P., Smith, J.U., Eds.; Springer: Heidelberg, Germany, 1996; pp. 237–246.
45. Liski, J.; Palosuo, T.; Peltoniemi, M.; Sievänen, R. Carbon and decomposition model Yasso for forest soils. *Ecol. Model.* **2005**, 189, 168–182. [[CrossRef](#)]



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