Summer school 2016 Soil & Water:

Leaf litter decomposition

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I) Introduction

Decomposition of plant litter is the most important source of nutrients for plants in many terrestrial ecosystems (Swift et al., 1979). Roughly 90% of global terrestrial plant production enters the dead organic matter pool making decomposition of plant material one of the most crucial processes in terrestrial and aquatic ecosystems (Hattenschwiler et al. 2005; Gessner et al. 2010). Controls on decomposition are therefore fundamental controls on important ecosystem processes like carbon (C) and nutrient cycling, primary productivity, community, structure and food web dynamics and ecosystem responses to environmental change (Hättenschwiler et al., 2005; Cornwell et al. 2008; Gobat et al. 2010). The relationship between biodiversity and ecosystem function has received a great deal of attention due to increasing global species decline (Loreau et al., 2002; Tilman et al., 2006; Meier and Bowman, 2008; Srivastava et al., 2009).

The litter decomposition process is mainly governed by three factors: i) environmental conditions such as mainly temperature and humidity (Gholz et al. 2000), but also UV radiation exposure or soil transport and infiltration; ii) leaf litter quality (chemical and physical characteristics of litter); and iii) composition and activity of the decomposer communities, including bacteria, fungi, arthropods (Santonja et al. 2015).

Leaf litter quality is known to be a major driver of species decomposition rates across biomes (Swift et al., 1979, Cadisch and Giller, 1997 and Cornwell et al., 2008). During early stages of decomposition, nutrients such as nitrogen and phosphorus, and water-soluble compounds have the largest effects, whereas at later stages, lignin is the primary determinant of decomposition dynamics (Berg and Staaf, 1980, Berg, 2000 and Rahman et al., 2013). As a consequence of their structural and chemical attributes, each species, when incubated in isolation, has a characteristic decomposition rate (“decomposability”, Pérez Harguindeguy et al., 2013). However, in nature, litter typically falls and decomposes in mixtures: litter layer derives from several species. In litter mixtures, the decomposition of one litter type may be influenced by the presence of other litter types, as reported in recent work on decomposition dynamics within multi-species litter mixtures in terrestrial and aquatic ecosystems. These studies investigating litter decomposition process along a gradient of plant species diversity showed two types of effects.

Firstly, additive effect with a decomposition rate of the litter mixture equal to the arithmetic mean of the respective component species decomposing alone, which means that there is no interaction among different litter types in multi-specifies litter mixtures.

Secondly, non-additive effect with a decomposition rate of litter mixture higher (synergistic) or lower (antagonistic) compare to the mean of the single species decomposition, which suggests that interactions among different litter types affect decomposition process (Santonja et al. 2015). Non-additive effects are mainly driven by the mixture components, such as the presence of Fast- or Slow-decomposing species, the magnitude of the difference in decomposability between the mixture components, the physical characteristics of litter that increase its water retention capacity, or the presence of recalcitrant compounds (Gartner and Cardon, 2004 and Hättenschwiler et al., 2005). Among the mechanisms proposed to explain synergistic effects on decomposition in heterogeneous mixtures nutrient transfer from litter of high quality to litter of lower quality has been

frequently invoked (McTiernan et al., 1997 and Kuziakov et al., 2000) but not always confirmed. Antagonistic effects have been mainly related to the presence of recalcitrant compounds such as lignin and phenolics compunds which may form resistant complexes with proteins (Hättenschwiler and Vitousek, 2000), inhibiting microbial growth and activities (Schimel et al., 1998). No general relationship exists between litter species diversity and MLML [mixed litter mass loss], as both synergistic and antagonistic effects may result in an overall neutral trend (Hättenschwiler et al., 2005).

Besides the identity of species that drives ecosystem functions, the number of species plays an important role for the stability of these ecosystem functions, as species-rich communities could have greater interspecific variation in responses to perturbation than species-poor communities (Santonja et al. 2015).

Predicting terrestrial ecosystem responses to global change is currently a major challenge for ecological research. Better understanding of how changing abiotic conditions (temperature or precipitation) can alter between-species interactions and consequently ecosystems processes could facilitate projections of ecosystem functioning in the context of climate change. However, the focus to date has been on ecosystem productivity, ad evolution in the litter decomposition process remains poorly informed. Here we report results from a litter bag decomposition experiment conducted in in the Sumava Mountains with single and two-species mixtures (Santonja et al. 2015).

We observed which type of mesofauna could be present in our samples, the decomposition rate of our samples. We are also looking for answers to these questions:

- Are there differences between the decomposition rate of two vegetation types (i.e. needles from coniferous and leaves from deciduous broadleaf trees)?
- Are there non-additive or additive effects on decomposition rates in litter mixtures?

II) Material and methods

a) Site description

Sampling was conducted in the mixed forest in Šumava foothills (49°4'55.789"N, 13°41'6.530"E, ca. 765 m above sea level) in the Czech Republic, South Bohemian region in mid-July. Annual site temperatures range between 5.0 and 6.0 °C, with the annual mean precipitation ranging from 750 to 800 mm. The forest is mostly dominated by deciduous species - alder (*Alnus glutinosa*), birch (*Betula pubescens*), willow (*Salix caprea*) with a mixture of spruce (*Picea abies*) and pine (*Pinus sylvestris*) and one individual beech (*Fagus sylvatica*). In the undergrowth, plants such as nettle (*Urtica sp*.), raspberry (*Rubus idaeus*) and some graminaceous plants prevail.

b) Litter decomposition experiment

The experiment was conducted through the use of the "litterbag" technique. The litterbags (also "samples") were placed into the field at the beginning of August. Before

starting the experiment of decomposing the litter, spruce needles (*Picea abies, Pinus sylvestris*) and leaves (*Fagus sylvatica*) were picked off from the trees at the surrounding sample study site. These senescent leaves were collected on a plastic sheet to avoid and prevent contamination with soil. Afterwards, the collected leaves and needles were divided into plastic bags, according to tree species and subsequently transported and stored in the freezing room at Jihočeská Univerzita, Přírodovědecká fakulta before starting the experiment.

All litterbags contained a total of approximately 5.0 g of plant leaves - of either spruce or deciduous species or mixed. Five grams of beech were placed into litterbags (leaves) (10 x 10 cm) for four replicates, five grams of coniferous species (needles) (*Picea abies, Pinus sylvestris*) for three replicates and five grams of homogenized mixed species (needles and leaves) (*Picea abies, Pinus sylvestris, Fagus sylvatica*) for three replicates as well. Mixed litterbags were filled for the evaluation of additive or non-additive effect of the decomposition (Santonja et al., 2015; Wardle et al., 1997). All litterbags were used for experiment of decomposition of primarily decompozers like mezo- (e.g. Acarina [Oribatids,Gamasids, Actinedida], Collembola) and microfauna (Fungi, Bacteria).

After filling, the litterbags were transported to Šumava foothills on the study site in the mixed forest and placed in the field unevenly on the soil surface. The litterbags were placed equidistantly next to each other under spruce, alder and birch on two square meters (2 m^2), under tree crowns on the ground where soil accumulation of litter is highest. Surrounding litter on the ground where litterbags were placed was relatively high, thus the litterbags were slightly impressed into it (about 20 %).

Litterbags were overlapped with adhesive tape around to prevent samples from mass losing e.g. by wind or some other disruption which might negatively affect the experiment. Samples were placed in the field on the study site in the beginning of July 2016. After five weeks on 11th September the litterbags were collected from the field, placed into a plastic bags to avoid contamination and transported to the laboratory at Jihočeská univerzita, Přírodovědecká fakulta. Afterwards, litter from the litterbags were shifted into heat-protect bowls, weighed (Servis VAH, Jiří Bláha s.r.o.) and dried (Drying oven Memmert) for 105° for 24 hours. Dried samples were weighed again (mass loss and water-holding capacity loss), results were evaluated.

In addition, the water content of fresh litter was tested, as was the average weight of 1 dry leaf, respectively needle and small bushel (K-control, D-deciduous, C-coniferous, C_B-coniferous bushel). The weight of 1 dry piece was calculated as $\frac{\boldsymbol{Weigt\ after\ drying}}{\boldsymbol{Number\ of\ pieces}}$, whilst the average weight of 1 dry piece was calculated as a mean of appropriate weights of 1 dry piece (leaves-KD1-3; needles of *Pinus sylvestris*-KC1-3; small bushels of *Picea abies*- KC_B1-3). The water content was calculated as percentage made from the mass loss, which is the loss of water in reality:

$$(\text{Water content } (\%) = \frac{(\text{Weight before drying} - \text{Weight after drying}) * 100}{\text{Weight before drying}}).$$

In a further step, the weight measured before and after the drying process was used to calculate the decomposition rate as follows:

$$\left(\text{Decom. rate} = \frac{(\text{mass before decom.} - \text{mass after decom.})}{\text{mass before decom.}}\right).$$

Upon calculating a negative decomposition rate, a further step was added in order to rectify the reconstruction of the unknown initial fresh mass by incorporating the dry mass of the field samples with the average water content into the following formula: $IFM = \frac{\text{WD}}{1-\text{WC}}$, whereby IFM is the initial fresh mass; WD is the weight of the dry mass of field samples; and WC is the water content. Finally, the average weight of 1 dry piece from laboratory experiment was used to calculate the unknown initial dry weight in field experiment according to the following formula: IW = NF*AW (IW - Initial dry weight in the field experiment; NF-Number of leaves in the field experiment; AW- Average weight of 1 dry piece from the laboratory experiment).

III) Results

For the evaluation of the field samples we should know the initial dry mass, which was derived from the results of laboratory experiment. These results accord the average weight of 1 leaf, needle and small bushel used in both laboratory and field experiments, and indicate variable content of water in both coniferous and deciduous litter (Tab. I).

Tab. 1 : Weight of samples before and after drying as well as average weight of 1 dry leaf, respectively needle and small bushel (K-control, D-deciduous, C-coniferous, C_B-coniferous bushel) with subsequent calculation of the fresh litter water content. Leaves-KD1-3; needles of *Pinus sylvestris*-KC1-3; small bushels of *Picea abies*- KC_B1-3).

	Number of pieces	Weight before drying(g)	Weight after drying (g)	Weight of 1 dry piece (g)	Average weight of 1 dry piece (g)	Water content (%)	Average water content	Average water content (%)
KD1	10	2,55	0,96	0,096	0,12	62.35%	0,61607	61,61
KD2	10	3,26	1,28	0,128		60,74%		
KD3	10	3,58	1,37	0,137		61,73%		
KC1	60	1,48	0,71	0,012	0,011	52,03%	0,511883	51,19
KC2	60	1,48	0,73	0,012		50,68%		
KC3	60	1,16	0,57	0,009		50,86%		
KC_B 1	10	3,36	1,61	0,161	0,152	52,08%	0,511676	
KC_B 2	10	2,82	1,41	0,141		50%		

KC_B3	10	3,17	1,54	0,154		51,42%		51,17

The average weight of 1 dry piece from laboratory experiment was used to calculate the unknown initial dry weight in field experiment (Tab. 2).

Tab. 2: Description of the field experiment (D-deciduous, C-coniferous, M-mixture) and results of calculated initial dry mass.

		Fresh mass		Dry mass		
	NUMBER OF LEAVES, NEEDLS AND BUSHELS	**LEAVES (g)**	**NEEDLES+ BUSHELS (g)**	**CALCULATED INITIAL MASS [g]**	**LEAVES (g)**	**NEEDLES + BUSHELS (g)**
D1	14	3,5	-	1,68	1,88	-
D2	18	2,85	-	2,16	2,04	-
D3	15	2,73	-	1,56	1,98	-
D4	13	2,93	-	1,56	2,03	-
C1	59+1B	-	3,41	0,801	-	2,47
C2	122+3B	-	4,07	1,798	-	2,4
C3	140	-	4,24	1,54	-	2,48
M1	67+1B	1,33	1,47	0,889	1,15	1,1
M2	68+1B	1,06	1,67	0,9	0,89	1,25
M3	63+1B	1,44	1,61	0,845	1,18	1,1

However, when the calculated initial dry weight was compared with dry weight of the field samples, it was realised that the initial dry weight was even smaller than the weight after drying, despite the fact that in reality a loss of weight was broad (Fig. 1, Fig. 2). The calculation of decomposition rate gave negative numbers, which means gain of mass. Considering the appearance of the leaves and bigger weight differences, the possibility of gaining weight because of decomposers (e.g. fungi) was rejected.

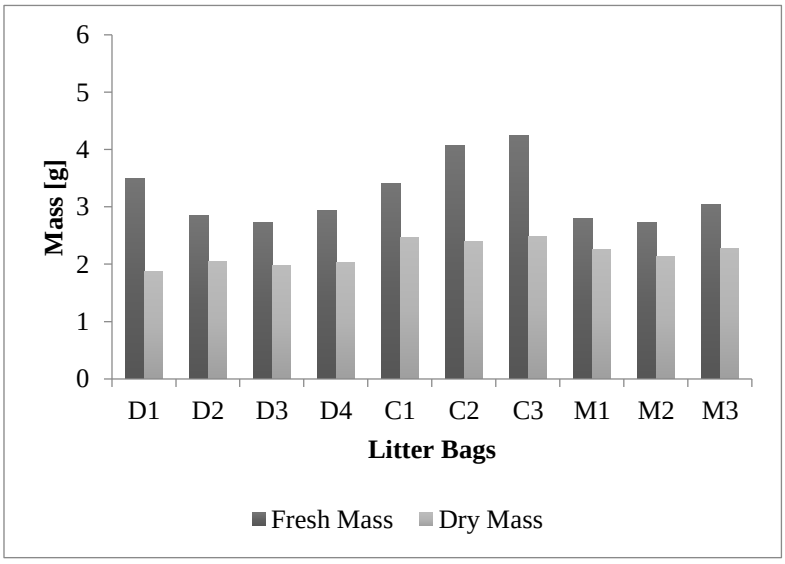

Fig. 1: The diference between weights of samples after 5 weeks in the field before(Fresh Mass) and after (Dry Mass) drying. We can see evident mass

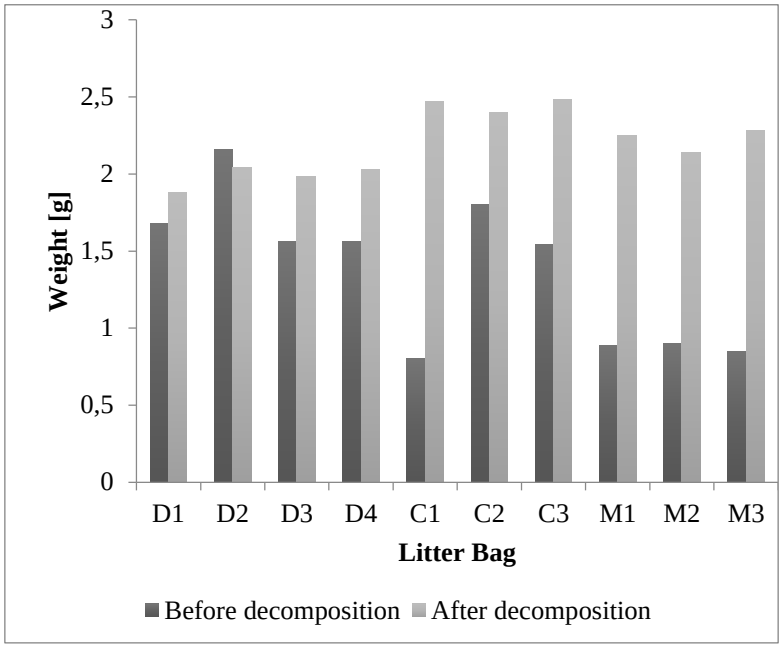

Fig. 2: Comparison of calculated initial dry weights (Before decomposition) and dry weights after 5 weeks of decomposition (After decomposition). Except D2, each sample gain weight after decomposition.

These results indicate some kind of mistake, and due to that, further calculations were done. Because of the appearance of the litter, almost no decomposition was presumed, on the other hand, the mass loss was presumed as a loss of water (Fig. 3).

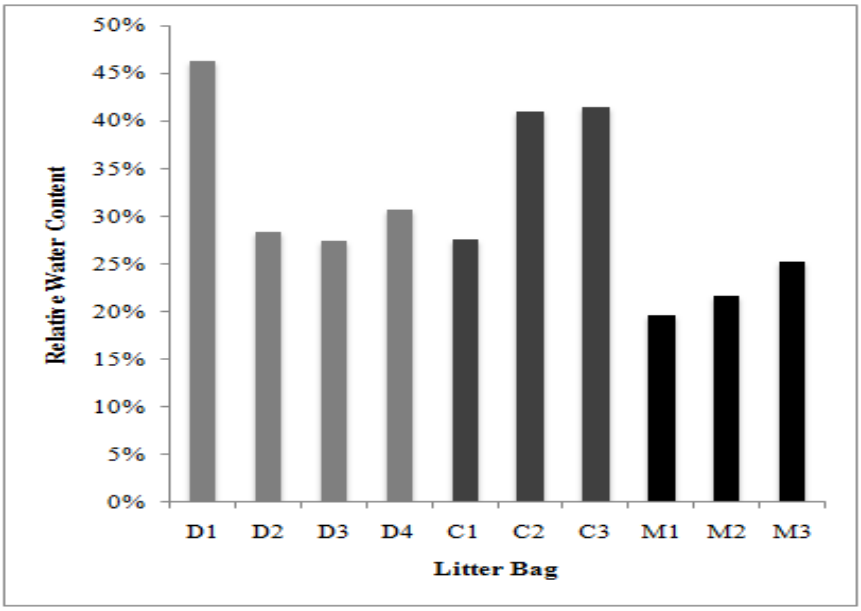

Fig. 3: Relative water content of field samples (D1-D4 for leaves, C1-C3 for needles, M1-M3 for mixed samples) in percentage.

According to the results shown in Fig. 3, if it is presumed that the mass loss of the field samples is just their water content, we can see its variability. To be able to test this possibility, the unknown initial fresh mass was reconstructed using the dry mass of field samples and the average water content from laboratory experiment.

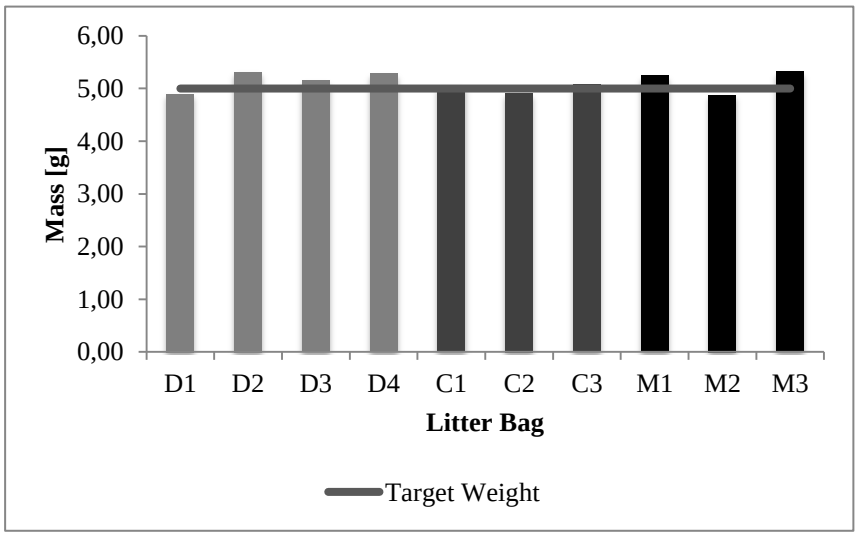

Fig. 4: Reconstructed fresh mass of decomposed samples, based on oven-dried samples and water content of fresh leaves**.**

The results of the reconstructed fresh mass are really closed to expected initial weight (5g) of the samples.

Fig. 5: Leaves of beech (*Fagus sylvatica*) before decomposition (on the left side) and after the time spent in the field (on the right side).

IV) Discussion / conclusion

The mass of the decomposed leaf litter in litter bags shows a decrease comparing the mass before and after the oven-drying, because of the water loss (Fig. 1). The reason for the wide variation of relative water content is unknown. There could be a species specific water content, however there is not only variation between deciduous and coniferous, but also within the single species (Fig. 3). This could be due to a difference of field conditions like exposition of sun light and rain, but because of the deficiency of information this cannot be examined. For future experiment the exact position of the litter bags in the field should be documented to allow the same treatment for all samples.

Due to the fact that the samples were not dried and weighed prior to being placed in the litterbags for decomposition, an estimation of the initial, pre-decomposition weight was made by weighing and comparing the fresh and dried mass of individual leaves, needles and bushels saved and preserved from the study site and projecting the average dry weight of each individual leaf, needle and bushel onto the fresh, post-decomposition samples. This method proved to be unreliable for two reasons: The estimated masses within the various litterbag groups (deciduous, coniferous, and mixed) displayed high variance and the dried, all but one (litterbag D2) post-decomposition samples had a *higher* mass than the pre-decomposition samples (Fig. 2). After the decomposition process, the litter mass was expected to decline instead of increase (Harmon et al. 2009). It is conceivable that the post-decomposition leaves were not entirely dry, since they were oven-dried at 105 °C for 24 hours, instead of at 60 °C for 48 hours (Santonja 2015). This would account for similar pre and post-decomposition masses, but not for the mass gain. In addition, the amount of samples used to base the average dry leaf, needle and bushel weight on could have been too small for a representative calculation of the variation in botanical morphology. Consequently, it is recommended to not only dry the samples thoroughly, but also to document the initial fresh and dry weight before placing the samples in litterbags for the decomposition process. Upon rejecting the values for the estimated initial, dry, pre-decomposition mass, an alternative calculation method was implemented.

By comparing the fresh and dried mass of the individual leaves, needles and bushels saved and preserved from the study site, the average, relative water content for all three litterbag groups was deduced and the values projected onto the mass of the dried, post-decomposition samples. These calculations show that the mass of the “fresh”, post-decomposition replicas is very close to the targeted 5.0 g (Fig. 4), implying that little to no decomposition took place. For several reasons, this was to be expected. Firstly, in light of the fact that water holding capacity plays a major role in decomposition rate (Santonja 2015) and weather conditions were hot and dry for the majority of the five weeks the samples were in the field (Diáková K, pers. communication), only little decomposition could have taken place. Secondly, the samples used in this experiment were cut directly from the respective trees, meaning they are not comparable to litter naturally abscised by trees, which contains less chlorophyll and fewer complex substances such as carbohydrates and

proteins (Smith & Cothren 1999). Lastly, the litterbags were placed in the field in mid-summer and for the short duration of five weeks, instead of in autumn, when most deciduous trees naturally abscise their foliage (DK Publishing et al. 2011). It is probable that the composition of decomposers in the ground changes over the course of the year and that the sample leaves were not subject to natural decomposer communities for a long enough period of time, thus affecting their rate of decay.

Consequently, there was no way to measure any additive or non-additive effects, because of the lack of data and the non-monospecifity of the coniferous litter bags. Normally a calculation based on Wardle (1997) would have been performed, followed by a statistical analyses based on an adapted student's t-test with the null-hypothesis 'the effect is zero.' A positive number would mean a positive synergistic non-additive effect, consequently a higher decomposition of one or more species in the mixture, whereas a negative number would result in a negative antagonistic non-additive effect with a lower decomposition of one or more species in the mixture (Gartner and Cardon 2004). As a suggestion for further investigations all used species in the mixture should be also as a monospecific sample in the field.

To summarize, a decomposition rate close to zero percent was to be expected, supporting the data behind our reconstructed "fresh", post-decomposition replicas. Observationally, the deciduous leaves underwent some decay, since – on the one hand – fungi were present on the leaves and – on the other hand – the leaves turned from green to brown (Fig. 5). However, the coniferous samples appear to have not been affected by decomposers. For future experiments, it is recommended to use naturally abscised leaves by, for example, installing leaf litter catching nets. Furthermore, the time of year should reflect natural leaf abscission conditions for the respective species used and the duration for the decomposition process extended. In addition, one could consider collecting weather data, such as temperature, humidity and precipitation from the study site.

To study more about leaf litter decomposition is necessary, because the decomposition allows the disposal of nutrient into its environment. Therefor it is a key driver for nutrient flow and the ecosystem functioning. As a consequence of climate and temperature change, a shift in plant communities occurs, which leads to a new mixture of plants and their leaf litter. In order to understand and respond to the impacts on the decomposition rate, and thus on the nutrient flow, it is fundamental to understand the factors that effect this process.

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