# The functional role of understorey herbs for litter decomposition in temperate forests

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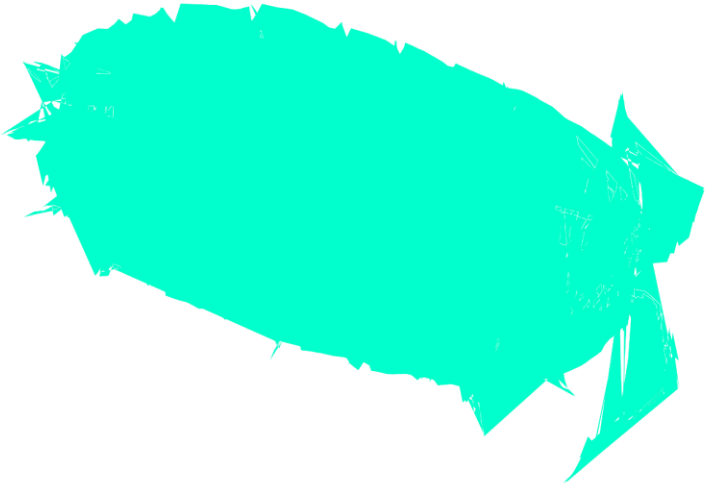

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# The functional role of understorey herbs for litter decomposition in temperate forests

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NATURAL RESOURCES

Dutch translation of the title:

De functionele rol van de kruidlaag voor de afbraak van strooisel in gematigd bos

Illustration on the cover:

Front: *Anemone nemorosa* L. (Adrian Swancar, Unsplash)

Back: Plot with herb layer presence and absence treatment in Aelmoeseneiebos, Gontrode (Bin Wang)

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# Summary

Litter decomposition is a vital process in forest carbon (C) and nutrient cycling, and it is known to be regulated by initial litter quality and climate variables. In forests, components like understorey vegetation are indispensable to ecosystem stability and ecological function. For example, the understorey layer may play an equally important role in regulating the soil microclimate as the overstorey tree canopy. Additionally, the understorey layer may also enhance forest floor litter quality, by providing a diverse and nutrient-rich litter to the forest litter layer, thus, directly and indirectly regulating litter turnover processes. However, traditionally, understorey plants (the 'understorey' in the following contest is a shorter synonym for 'the forest understorey herb layer') have been considered as a constraining factor in silviculture, as the understorey vegetation competes with tree seedlings for resources, i.e., light, nutrients, and water. In recent decades, there has been a growing recognition that the understorey layer plays an important role in driving ecosystem processes and functioning. However, we still know relatively little about the effects of the understorey layer on the decomposition of tree litter, which forms the biggest natural source for C and nutrients in the topsoil of most forests. This thesis aimed to unravel the relative importance of the functional role of understorey herbs for forest litter decomposition, by testing its effects on the soil microclimate, its further impact on litter decomposition, and by testing its mixture (direct) effects on the decomposition of tree litter in temperate forests.

We conducted an understorey removal field experiment and a mesocosm experiment with understorey plant species to investigate the regulatory effects of the understorey layer on litter decomposition, using green tea and rooibos teabags as standardized litter. We measured effects of understorey removal and percentage cover on the soil microclimate (temperature and water content) and litter decomposition. We found that the removal of the understorey significantly increased mean soil temperatures in the topsoil by 0.21 ± 0.03°C in the beginning of the incubation. The understorey layer could significantly buffer hot and cold thermal extremes at crest or trough of temperature changes, respectively, as temperature increases with fluctuations. Similarly, the soil moisture content was on average 6.32 ± 0.81 % higher in understorey removal than in the control plots. Additionally, during our short-term incubation, there was no significant difference in mass loss and moisture content of both green tea and rooibos tea between of the understorey treatments. Nonetheless, the percentage cover of the understorey vegetation, under natural conditions or under the environmental enrichment conditions, was negatively correlated with mass loss of both green tea and rooibos tea.

To test for possible mixture effects of understorey herbaceous litter on the decomposition of overstorey tree species litter, we combined the (nitrogen and phosphorus rich) litter from two herbaceous species (*Anemona nemorosa* and *Aegopodium podagraria*) with each of five tree species (*Fagus sylvatica* L., *Quercus robur* L., *Acer pseudoplatanus* L., *Fraxinus excelsior* L., and *Tilia platyphyllos* Scop.). These tree species represent a gradient in litter quality. Two herbaceous litter addition experiments were performed, one under laboratory conditions and the other *in-situ* in an ancient temperate mixed deciduous forest in northern Belgium. We found that the addition of *A. podagraria* litter on top of tree litter led to a higher litter moisture content, abundance of soil fauna, and enriched litter nutrient content, resulting in a constant synergistic (positive) effect on fresh tree litters in laboratory conditions and on the litter decomposed for half a year in the field. The addition of *A. nemorosa* litter had, however, antagonistic (negative) effects on recalcitrant tree litter, despite it also significantly increased the abundances of soil fauna.

Our findings highlight that, despite the fact that the overstorey canopy is commonly identified as the major driver for variation in the forest microclimate, the understorey herbaceous layer can also play an important role, by regulating soil microclimate when it is alive, or by providing nutrient-rich litter when it is decomposing. However, contrary to our expectations, the warmer and wetter soil environment caused by understorey removal did not stimulate litter decomposition, instead, the percentage cover of the understorey layer showed a consistent negative correlation with litter decomposition. In addition, the litter inputs from the understorey plant community can have a substantial influence on the decomposition of tree litter, i.e. mixture effects that operate through nutrient leaching and changing the community of soil biota. As an overlooked litter inflow to the forest litter layer, the presence of herb litter on top of tree litters improves microenvironmental conditions for decomposition of tree litters and increases soil fauna abundance in the mixture systems. Nonetheless, the most likely explanation for mixture effects of herb litter on tree litter should be attributed to the transfer of nutrients from herb litter towards the tree litter. Together, these experimental results imply that the understorey plant community contributes to the regulation of the soil biotic and abiotic environment, with possibly important consequences for the nutrient and carbon dynamics in forest ecosystems.

# Samenvatting

Strooiselafbraak (decompositie) is een vitaal proces in de koolstof- en nutriëntencycli van bossen. Decompositie wordt beïnvloed door de initiële strooiselkwaliteit en door klimaatsfactoren. In bossen zijn ook andere componenten zoals de kruidlaag onmisbaar voor ecosysteemstabiliteit en ---functies. De kruidlaag kan bijvoorbeeld een even belangrijke rol spelen in het reguleren van het bodemmicroklimaat als de boomlaag. De kruidlaag kan ook de strooiselkwaliteit verbeteren, door een meer divers en nutriëntenrijker strooisel te voorzien en zo direct of indirect afbraakprocessen te reguleren. In het verleden werd de kruidlaag echter vaak als een hinderende factor voor de bosbouwpraktijk beschouwd omdat de soorten in competitie voor licht, nutriënten en water treden met zaailingen van bomen.. In de laatste decennia is er echter een groeiend begrip ontstaan dat de kruidlaag een belangrijke rol speelt in het sturen van een breed spectrum aan ecosysteemprocessen en -functies. Toch weten we relatief weinig over de effecten van de kruidlaag op de afbraak van strooisel. Strooisel vormt de grootste natuurlijke hulpbron van koolstof (C) en nutriënten in de toplaag van de meeste bossen. Deze thesis onderzoekt het relatieve belang van de functionele rol van kruidachtige planten voor strooiselafbraak, door de effecten van de kruidlaag te testen als regulator van het bodemmicroklimaat, diens impact op strooiselafbraak, alsook mengingseffecten van kruidlaagstrooisel en boombladafval in gematigde bossen.

We voerden een experiment met verwijdering van de kruidlaag uit, en een mesocosm experiment met kruidachtige bosplanten om te onderzoeken wat het regulatie-effect is van de kruidlaag op strooiselafbraak, waarbij we gebruik maken van groene thee en rooibos thee als gestandaardiseerd strooisel. We onderzochten effecten van kruidlaagverwijdering en kruidlaagbedekking op het bodemmicroklimaat (temperatuur en bodemvochtgehalte) en strooiselafbraak. We vonden dat de verwijdering van de kruidlaag tot een significant hogere gemiddelde bodemtemperatuur leidde (+0.21 ± 0.03°C) bij aanvang van het experiment. De kruidlaag kon op een significante wijze de warme en koude thermale extremen bufferen. Op een gelijklopende manier nam het bodemvochtgehalte toe met 6.32 ± 0.81 % in de behandeling met kruidlaagverwijdering in vergelijking met de controle. Gedurende de kortetermijnincubatie was er geen verschil in massaverlies en vochtgehalte van zowel groene thee en rooibosthee tussen de proefvlakken met en zonder aanwezigheid van de kruidlaag. Desalniettemin was de bedekkingsgraad van de kruidlaag, zowel in natuurlijke condities als veroorzaakt door veranderingen in de omgeving, negatief gecorreleerd met het massaverlies van zowel groene thee als rooibosthee.

Om de mengingseffecten te testen tussen het strooisel van kruidachtige planten en dat van boombladafval hebben we het (stikstof- en fosforrijke) strooisel van een van twee kruidachtige planten (*Anemona nemorosa* en *Aegopodium podagraria*) apart vermengd met strooisel van vijf boomsoorten *(Fagus sylvatica L., Quercus robur L., Acer pseudoplatanus L., Fraxinus excelsior L.,* en *Tilia platyphyllos Scop.*). Deze boomsoorten bepalen een gradiënt in strooiselkwaliteit. Twee verschillende strooiseladditie-experimenten werden opgezet, één in labocondities en één *in-situ* in een gematigd gemengd oud bos in Noord-België. We vonden dat het toevoegen van *A. podagraria* strooisel bovenop boombladafval leidde tot een hogere bodemvochtgehalte, abundantie van bodemfauna, en rijker nutriëntengehalte van het strooisel. Deze effecten leidden tot een eenduidig synergistisch (positief) effect op zowel vers boombladstrooisel in labocondities als strooisel dat een half jaar in het bos lag. De toevoeging van *A. nemorosa* strooisel had, integendeel, een antagonistisch (negatief) effect op het recalcitrante boombladafval, ondanks een toename in de abundantie van bodemfauna.

Onze bevindingen benadrukken dat, ondanks het feit dat de boomlaag beschouwd wordt als de sterkste stuurfactor in het bosmicroklimaat, de levende kruidlaag hier ook een belangrijke rol speelt, door het bodemmicroklimaat te regelen. Tegen onze verwachtingen in veroorzaakten warmere en vochtigere bodems in het experiment met kruidlaagverwijdering geen verhoogde strooiselafbraak. In de plaats daarvan vonden we een eenduidig negatieve correlatie tussen bedekkingsgraad van de kruidlaag en strooiselafbraak. Bovendien had de strooiselaanvoer van de kruidlaaggemeenschap een belangrijke invloed op de decompositie van boombladstrooisel, waarbij mengingseffecten optraden via nutriëntenuitloging en diens invloed op de bodemfauna. Als een aanvoer die nog te vaak genegeerd wordt, vonden we dat de toevoeging van kruidachtig strooisel de micro-omgeving kon verbeteren en de bodemgemeenschappen in de strooiselmengingen kon versterken. Het mengingseffect van kruidlaagstrooisel op boombladstrooisel kan echter vooral toegeschreven worden aan de transfer van nutriënten van het strooisel van de kruidachtigen naar het strooisel van de bomen. Tezamen impliceren deze resultaten dat kruidlaaggemeeschappen bijdragen aan het reguleren van bodembiota en de abiotische omgeving, met mogelijks belangrijke gevolgen voor nutriënten- en koolstofdynamieken in bosecosystemen.

# Abbreviations

Abbreviations

| | |
|---|---|
| AFDM | Ash free dry mass |
| PC | Principal component |
| PCA | Principal components analysis |
| NMDS | nonmetric multidimensional scaling |
| SEM | separate structural equation models |
| ANOVA | analysis of variance |
| Oligo | Oligotrophic soil |
| Meso | Mesotrophic soil |
| Eu | Eutrophic soil |
| T | Temperature treatment |
| L | Light treatment |
| LU | Land use treatment |
| $\text{Herb}_{(A)}$ | Above addition |
| $\text{Herb}_{(B)}$ | Below addition |
| TS | the tree species |
| D | Detritivores |
| H | Herb litter |
| +Hsp | Spring herb litter addition |
| +Hsu | Summer herb litter addition |
| +Hsp+Hsu | Spring and summer herb litter addition |

Chemical compounds

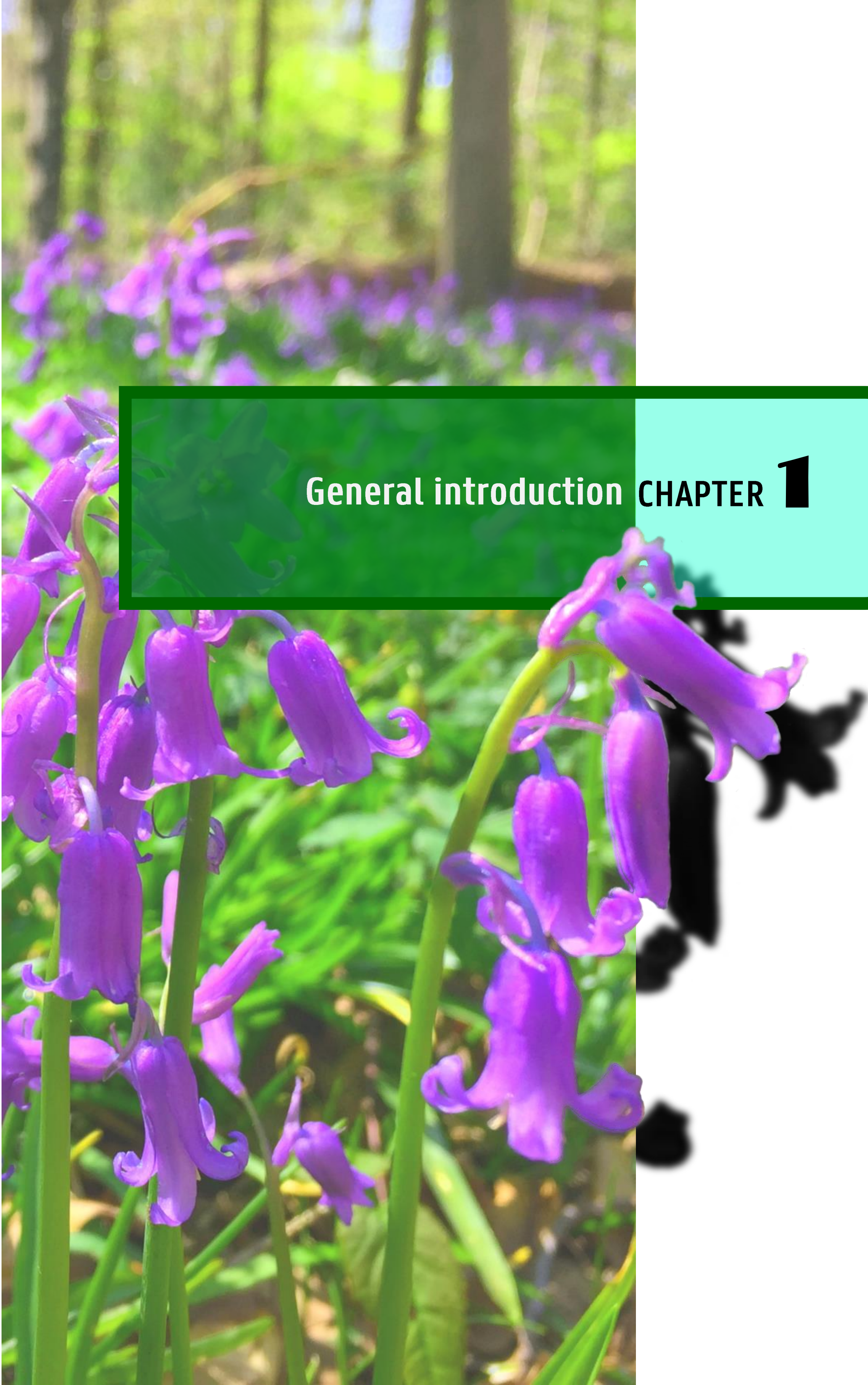
| | |
|---|---|
| C | Carbon |
| N | Nitrogen |
| TC | Total carbon |
| TN | Total nitrogen |
| TP | Total phosphorus |
| TK | Total potassium |
| TCa | Total calcium |
| TMg | Total magnesium |

Symbols

| | |
|---|---|
| *k* | Decomposition rate according to Olson's single exponential model |
| *h* | Shannon-Wiener index |
| *p* | Significance of statistical test |
| *r* | Pearson correlation coefficient |
| *F* | ANOVA test statistics |
| *t* | Test statistic (t-value) |
| *df* | degrees of freedom |
| $\chi 2$ | chi-square statistic |

# CHAPTER 1

# General introduction



## 1.1 Litter decomposition.

Decomposition of plant residues provides the primary source of soil organic carbon (C) and mineral nutrients for biological activity in most terrestrial ecosystems (Parton *et al.* 2007). The annual inflow of dry matter from tree and understorey vegetation (the ‘understorey’ in the following contest is a shorter synonym for ‘the forest understorey herb layer’) was estimated to be 20-40 Pg C yr$^{-1}$ (0.77 – 6.6 t C ha$^{-1}$ yr$^{-1}$) in temperate forests, with more than 70% composed of leaf tissue (Bray & Gorham 1964; Matthews 1997; Krishna & Mohan 2017). Litter decomposition is an integrated and lengthy process that breakdown of organic productions into their prime constituents ($CO_2$, $H_2O$ and mineral components), which involves numerous unordered physical, chemical, and biological pathways (Fig 1.1) (Aerts 1997; Krishna & Mohan 2017). For instance, forest litter is broken into (macro) fragments by natural forces, such as winds, rainfall, or animal activities, then the fragments are further decomposed into smaller stable aggregates, organic matters, or molecules through decomposers gut processing or via catalyzation by soil enzyme (Gallo *et al.* 2004). Hence, the physicochemical properties of litter and its biotic and abiotic environment are the predominant drivers of litter decomposition at local and large spatial scales (Parton *et al.* 2007; Cornwell *et al.* 2008; García-Palacios *et al.* 2013). We therefore subsequently describe in more detail the role of litter quality, the abiotic environment (mostly climate), and the biotic community that is involved in decomposition (see also Fig. 1.1).

In spite of the complexity of the decomposition process, it still can be roughly classified into three simultaneous processes depending on the sequence of degradation of components in litter: (i) the leaching of soluble compounds into the soil mostly happens in the early stage of litter decomposition, (ii) high N concentrations gradually suppress the degradation of recalcitrant substances, such as lignin, cellulose, phenols and tannins, and (iii) the humus-near stage where the remnants are humified by microorganisms and soil enzymes (Berg 2014). Hence, the litter chemical composition (referring to the proportion of labile components, e.g., C: nitrogen (N) ratio or lignin: N ratio) is likely to be very important for decomposition rates (Coûteaux *et al.* 1995). To date, litter quality characteristics are most useful in describing litter decay rates, as these characteristics differ with the plant tissues (leaves, stems, roots, bark, flower, and seed) and plant species. For example, in temperate forests, tree species can be ranked from high (e.g., ash *Fraxinus excelsior*) to low-quality litter (e.g. beech *Fagus sylvatica*), among others, based on their C:N ratio and lignin content (Hättenschwiler & Gasser 2005; Vesterdal *et al.* 2008; Jacob *et al.* 2010). In this respect, litter quality generally explains most of variation in decomposition rates (Limpens & Berendse 2003; Cleveland *et al.* 2014; Keiser & Bradford 2017).

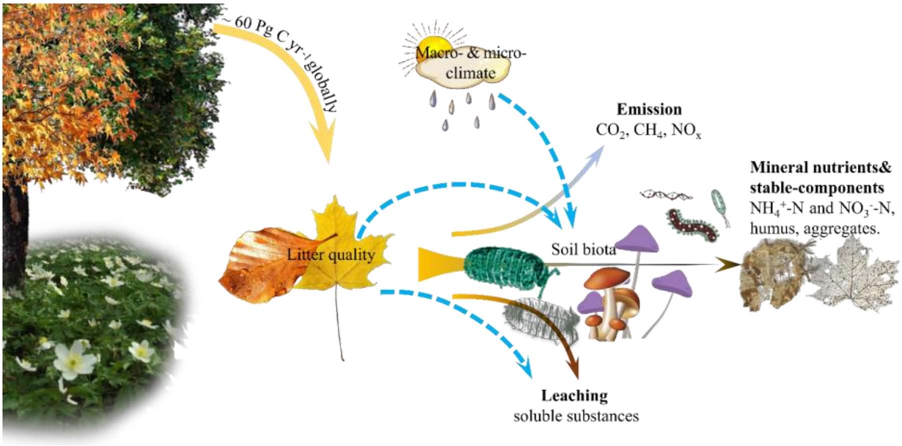


**Figure 1.1**. Conceptual diagram depicting the factors affecting litter decomposition. Litter decomposition is a lengthy process, with mass loss declining exponentially with time. Soil biota consumption (e.g., soil fauna, fungi, and bacteria), C and N emissions, and substances leaching are the main reasons leading to mass loss. And, environmental factors and litter physico-chemical traits regulate the decomposition rates via affecting the activity of soil biota. The solid arrows present corporeal processes, the dashed arrows represent suppositional factors.

The dominant role of litter quality in the prediction of litter decay rates would be, however, compromised in extreme environments, where environmental variables (temperature and moisture) rule key ecosystem functions related to decomposition, including microbial and enzymatic activity. Thus, the climate variables will modulate litter decomposition rates (Aerts 1997; Murphy *et al.* 1998; Shaw & Harte 2001). For example, Parton *et al.* (2007) identified that the climate is the best predictor of decomposition rates at the global scale and can explain about 70% of the variability in litter decomposition. However, this is not the case at regional or local scales, as more and more field and laboratory studies continue to provide evidences that the response of litter decomposition to environmental changes were not always positive. For example, Xu *et al.* (2015), De Long *et al.* (2016), and Petraglia *et al.* (2019), all reported that macroscopically increasing temperature alone did not (or weakly) stimulate litter decomposition. The litter decomposition is not only being regulated by the macroclimatic conditions, but also a consequence of (near-surface) soil microclimate which is generally being regulated by forest vegetation structures (e.g., forest canopy, shrub layer, understorey vegetation) in forests. The soil temperature under the understorey layer was reported to decrease ~1 °C compared to understorey removal (Xu *et al.* 2010; De Long *et al.* 2016), because the light and solar radiation is weakened or reflected by the canopy structure of understorey plants (Zellweger *et al.* 2019). The decreased soil temperature caused by the understorey layer might, therefore, create a cooler soil microclimate for litter decomposition than under an understorey removal treatment (De Long *et al.* 2016). For example, warming generally increases understorey productivity and biomass (De Frenne *et al.* 2015), which, in turn, could reduce decomposition rates due to soil cooling via increased shading (Myers-Smith & Hik 2013).

Litter quality and climatic factors play a regulatory role that may influence the decomposition rates, much like petrol quality and the oxygen environment to car speed. But what really drives the decomposition forward are the decomposer communities. They are the engine of decomposition (Glassman *et al.* 2018) being directly or indirectly involved in about 90% of organic matter decomposition in natural ecosystems (Swift *et al.* 1979). Decomposer communities, e.g., soil microbial and fauna, can either directly drive decomposition by ingesting and digesting along the passage through the gut (Zimmer *et al.* 2005), or indirectly by breaking litter into smaller size that leach and are attacked by smaller soil organisms, e.g., microbiological and enzymatic degradation (Frouz 2018). The contribution of decomposers to decomposition rates is difficult to predict in natural systems, despite the decomposers being the ultimate successor for (almost all) the energy stored in litter. There are many possible reasons for this. First of all, the effect of decomposers on litter decomposition is species-specific, due to the feeding preferences of taxa and populations, for example, phytophages feed on fresh leaves while detritivores feed on fallen leaves (Pontégnie *et al.* 2005; Xu *et al.* 2020). Secondly, decomposers also have different climate regimes (Keiser & Bradford 2017), for example, some soil fauna prefer to be active in dry environmental conditions, while some others like moist environment to settle in. Moreover, community characteristics, e.g., abundance, diversity, gender, and body size, are also influence on prediction accuracy of decomposition rates in the model (Schon *et al.* 2008). Thirdly, the function of litter to decomposer changes over time, since the decomposers do not only treat litter as a food source, but also as habitat or hunting-ground (Hättenschwiler *et al.* 2005).

## 1.2 Litter mixture effects

The litter layer is generally composed of decomposing parts of multiple species of trees, shrubs, and herbs, which may exhibit mutual influences during decomposition. Interactions between the litter of different species in mixtures may affect decomposition rates and nutrient dynamics during litter decomposition, resulting in non-additive effects, i.e., accelerated (synergistic) or reduced (antagonistic) decomposition rates, compared with those estimated from monoculture species (“litter mixture effects”; Figure 1.2) (Gartner & Cardon 2004; Schweitzer *et al.* 2005; Liu *et al.* 2020). There are three principal mechanisms behind these non-additive effects: (i) it is widely accepted that litter mixture effects are partly caused by the changes in decomposer community, e.g., soil microorganisms and fauna, due to their litter feeding preferences (Chapman *et al.* 2013; Wu *et al.* 2014; Fontana *et al.* 2019), (ii) the transfer of inhibitory litter constitutes and nutrients between mixtures can also lead to non-additive effects (Schimel & Hättenschwiler 2007; Lummer *et al.* 2012; Bonanomi *et al.* 2014), and (iii) leaf litter structural variation can modify micro-environmental conditions in litter mixtures resulting in increased or decreased decomposition rates (Vivanco & Austin 2008). Below we elaborate on these three mechanisms.

Different species produce litters with different chemical compositions. This leads to distinct palatability and digestibility that drives selective feeding of different decomposers, e.g., detritivores (Brandl *et al.* 2003; Cornelissen *et al.* 2004). Different litter will thus partly build up different decomposer communities, according to the feeding preference of decomposers (Hättenschwiler & Gasser 2005; Mcguire & Treseder 2010). According to this mechanism, mixing of two or more species would also attract the different associated decomposer communities to the mixtures system, resulting in an increase of the abundance and diversity of decomposers (Wu *et al.* 2014; Xiao *et al.* 2020). The increase of decomposer communities is expected to directly promote litter decomposition rates, by increasing litter consumption, or indirectly by stimulating the decomposition rates through increasing leaf surface structure (which benefit to microbes). However, a growing body of evidence suggests that the decomposer communities in litter mixtures are not simply the sum of the communities in the individual litter, due to overlap in soil fauna and microbial taxa (Hättenschwiler *et al.* 2005; Jiang *et al.* 2013), because of increased habitat heterogeneity encouraging some other decomposer species to settle in (Gessner *et al.* 2010), or because the new litter source(s) might contain deterrent components for some of decomposers (David & Handa 2010; Quadros *et al.* 2014). Moreover, the decomposition of litter mixtures does not always benefit from the combination of different decomposer communities, due to the trophic structure also being affected by the mixture (Melguizo-Ruiz *et al.* 2020). For example, total abundance of nematodes in *F. sylvatica*, *Tilia cordata*, and *Fraxinus excelsior* litter mixtures was remarkably higher than in what would be expected based on abundances in monocultures of the different species (Cesarz *et al.* 2013), of course, the predators, like bacterial and fungal feeders, also contributed to the increase in total abundance, which might, in turn, restrict the activity of microbial decomposer communities (Gessner *et al.* 2010).

The transfer of nutrient between different litter types in mixtures is expected to be different from those observed in monocultures, because of greater nutrient diversity in mixtures than in monocultures (Laganière *et al.* 2010; Xiao *et al.* 2020). Compared with low quality litter, high quality litter is generally more blittable and leachable because of high contents of water-soluble substances and unshielded holocellulose, which allows the nutrient transfer between leaves in mixture systems to go dominantly from higher to lower quality litter (Lummer *et al.* 2012; Bonanomi *et al.* 2014). A physical transfer of nutrients, e.g., via litter fragments and leaching of water-soluble substances, is generally associated with forest hydrological processes. For example, the nutrients and fragments from high-quality litter can be gravitationally transferred with water flow when the high-quality litter lays on top of the low-quality litter, on the contrary, the nutrients can be passively lifted with capillary water rise when the high-quality litter lies underneath the low-quality litter. The passive nutrient transfer could also, on the other hand, deliver some negative substances, e.g., inhibitory compounds for decomposers can be also transported during passive transfer of nutrients (Lummer *et al.* 2012). In addition to the passive nutrient transfer, there is also a biologically-driven nutrient transfer, e.g., through fungal mycelia networking, with definite nutrient and clear transportation direction, for example, fungi directly take-up N (or even fatty acids, amino acids or vitamins) from nutrient-rich litter to nutrient-poor litter (Lummer *et al.* 2012). That is, fungi growing in nutrient-poor litter can extend their hyphae to alternative nutrient-rich litter, redistributing the assimilated nutrients within their hyphal network (Gessner *et al.* 2010). Consequently, the nutrient status and decomposition rates of low-quality litter may be improved through a combination of physical and biological nutrient transfers.

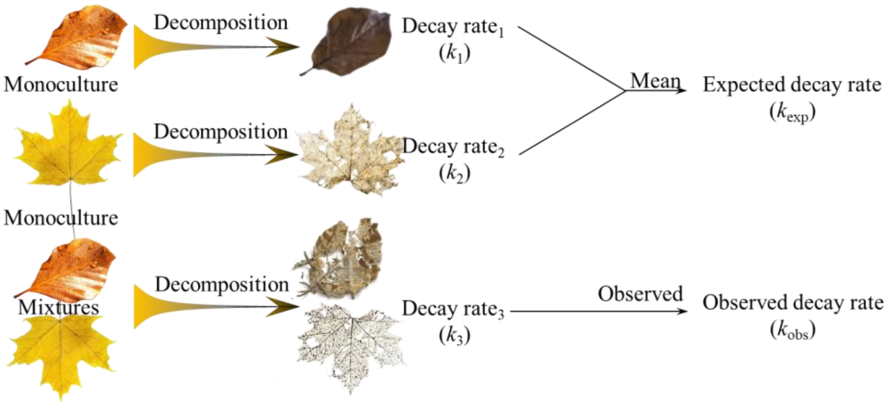


**Figure 1.2**. Conceptual diagram depicting non-additive effect. The species shown are two common temperate deciduous broad-leaved tree species, beech and maple. Decay rate represent litter decomposition rates, which is generally calculated by fitting the fraction of litter mass loss with sampling times according to the Olson's exponential model ($\ln(m_t/m_0) = -kt$). The expected decay rate is the decay that one would expect based on monospecific litter decomposition rates and in the absence of interactive effects between the litter types (i.e. it is an average of the monospecific rates). The mixture litter shows a synergistic effect if $k_{exp}$ is smaller than $k_{obs}$, on the contrary, it is an antagonistic effect if the $k_{exp}$ is smaller than $k_{obs}$.

Mixing litter with different quality substances can not only encourage the decomposers and enrich the nutrient status, but can also improve microenvironmental conditions in the mixtures. Most of high-quality litter generally contains higher hydrophilic substances (e.g., protein, starch and cellulose) that improve its water retention capacity. Therefore, mixing low quality litter with high quality litter may enhance decomposition rates by creating a more favorable habitat and food source for decomposers in the decomposing environment (Hättenschwiler *et al.* 2005). Wardle et al. (2003) were the first to report on the improved microenvironment theory in litter mixtures, as they found the slowly decomposing litter, feather mosses, stimulated decomposition rates of tree litter because of their ability to enhance the moisture retention in the litter layer.

## 1.3 The functional role of understorey herbs

The forest understorey herb layer contains vascular plants (woody and non-woody) below a threshold height of ca. 1 m (Gilliam, 2007), and is generally characterized by high nutrient and water content (Melillo *et al.* 1982), are traditionally considered as an unimportant 'step-over' vegetation layer for forest management (Gilliam 2007). Despite being less dominant in terms of biomass compared to the overstorey trees, species in the understorey layer consume a considerable portion of the available nutrients, enhance water evaporation from the topsoil, and compete for light with tree seedlings so that there is an important effect on the long-term forest dynamics (De Lombaerde *et al.* 2020). Furthermore, in temperate forests, the understorey layer is known to contain most of the plant biodiversity (Gilliam *et al.* 2016) and may, through its functional variation, significantly shorten the nutrient cycling period because of high nutrient uptake, short growing, and rapid decomposition. In addition, the understorey layer also plays a remarkable role in belowground microclimate regulation.

In forest ecosystems, the tree canopy plays an essential role in thermal and hydrological buffering, thus, regulating the microclimate in the forest interior. Zellweger et al. (2020) recently reported that the overstorey canopy can buffer temperature extremes up to 2 °C in temperate and boreal forests(Zellweger *et al.* 2020). However, before the light, heat, or rainfall reaches the forest floor, it may be further be intercepted by the understorey layer. As a large fraction of through falling light and throughfall is intercepted by the herb canopy (Gräff *et al.* 2015), less solar radiation and rainfall penetrates into the forest floor, further altering the microclimate at forest floor level where litter decomposition takes place. For example, some previous studies have shown that understorey vegetation removal could create on average an 1 °C warming of the soil environment (Matsushima & Chang 2007; He *et al.* 2020b). Moreover, the understorey layer does not only block the thermal and hydrological sources, but also declines the soil water content by increasing water evaporation via transpiration by leaves, which consequently leading to a lower water availability. The intensity of the microclimatic regulation by the understorey layer may consequently influence further biological processes such as litter decomposition, as temperature and moisture are the key factors for biochemical processes in soil (see section 1.1).

Apart from its regulatory effect on microclimate, an additional ecological function of the understorey layer is that it provides a broad array of often highly palatable and nutritious food resources to herbivores because of the often high litter quality (e.g., high nutrients and water but low lignin content) compared to the overstorey tree species (Landuyt *et al.* 2019). Furthermore, species in the understorey show wide variation in phenology, from vernal species that provide litter already in early summer to summer species that experience senescence in autumn. For example, the aboveground part of *Anemone nemorosa* generally shreds after spring, while *Aegopodium podagraria* is generally shredding after summer or early autumn (Jagodziński *et al.* 2016). The species in the understorey thus provide litter inputs throughout the growing season and are therefore possibly important for maintaining biotic activity in temperate forest, i.e. producing residues to the soil more continuously compared to the tree litter that is mainly produced in autumn. This seasonal input of litter largely fills a “nutrient gap” at the forest floor, as most of high-quality tree litter that has fallen in the last autumn will be mostly decomposed (at least the most valuable part) during winter and spring.

Given that the litter of understorey herb species often differs considerably from tree litter, in quality, quantity, and phenology, there are good reasons to assume it will also lead to litter mixture effects when mixed with litter from trees. First of all, the high content of hydrophilic substances in herb litter would be helpful for moisture maintaining in the mixtures layer (Xiong *et al.* 2008). Second, the high concentration of nutrients, water-soluble substances, and unshielded holocellulose would make the transfer of nutrients easier compared to tree litters, either through nutrient leaching or fungi hyphal networking, as the herbs (senescence in late spring of summer) generally fall on top of decomposing tree litter (senescence in autumn of the previous year). Third, as a nutritious, palatable, and digestible food resource in the forest, herbaceous litter would be attractive to the decomposer community, possibly causing a distinct decomposer community to settle in. This could directly or indirectly affect turnover rates of tree leaves in the mixtures (Chomel *et al.* 2016). For example, He *et al.* (2020a) and Milcu *et al.* (2006) found that the presence of herb litter in mixture with tree litter kept a continually higher microbial biomass nitrogen (MBN) content and collembola density compared to tree monoculture, resulting in synergistic non-additive effects. Based on the mechanisms that underlie mixture effects in tree litter, mixing herb litter with tree litter is expected to have positive effects on tree litter decomposition, unless the herb litter contents some deterrent components, e.g., tannin or other inhibitory components.

## 1.4 Research gap, objectives and outline of the thesis

### 1.4.1 Research gap

Forest understorey vegetation is an important component of temperate forest ecosystems, having a disproportional (based on its biomass) effect on forest ecosystem functioning, such as biodiversity conservation, regulating forest regeneration, hydrologic resource allocation, and carbon storage (Thrippleton *et al.* 2016; Wang *et al.* 2016; Landuyt *et al.* 2019). The functional role of the understorey layer for C and nutrient turnover processes is, however, rarely studied, despite the potential strong effects of (alive) understorey vegetation to soil microclimate, much like the overstorey tree canopy does (via thermal, hydrological, and optical buffering). There are good reasons to assume herb – tree litter mixtures would show important litter mixtures effects, through similar mechanisms that also cause mixture effects of different tree litter species. And finally, the ephemeral herbs in temperate forests senescence and fall in different moments (e.g., later spring and summer) of the year compared to the tree species, which could further cause non-additive effects on decomposition rates of tree litter.

### 1.4.2 Objectives

The main aim of this study was to **unravel the possible effects of the understorey herb layer and its litter on tree litter decomposition**. In our study, we used two main approaches: (i) testing regulatory effects of understorey vegetation (the understorey is generally defined as the vegetation below a threshold height of ca. 1 m, while in this thesis, the term “understorey” mostly represents the understorey herb layer, as it is the most abundant and functional constituent of the understorey layer) on the soil microclimate, and how this affects decomposition of standardized litter types (green tea and rooibos tea; Keuskamp *et al.* 2013; Djukic *et al.* 2018), and (ii) testing mixture effects with adding understorey herb litter (*A. nemorosa* and *A. podagraria*) above tree litter from one of five European common tree species (common beech (*Fagus sylvatica* L.), pedunculate oak (*Quercus robur* L.), sycamore maple (*Acer pseudoplatanus* L.), ash (*Fraxinus excelsior* L.), and large-leaved lime (*Tilia platyphyllos* Scop.)). With this combination of approaches, we investigated different functions of the understorey layer, differentiating between its effect as standing vegetation (i.e. when the herb species are alive; “regulatory function”) and its effect after withering and falling on the forest floor (i.e. when it adds on the litter layer; “mixture function”).

Our first main question regarding the **regulatory function** was: How does the understorey herb layer buffer macroclimate and thus regulates soil microclimate (e.g., soil temperature and moisture content) in temperate forest (Fig. 1.3 a). And then, how this regulated microclimate will affect decomposition processes of standardized litter (tea bags) in the soil. Specifically, we compared the soil microclimatic conditions and tea bag mass loss under herb layer presenting and absenting treatments (Fig. 1.3 b). Then, we examined direct and understorey-mediated indirect effects of (human-induced) environmental changes on litter decomposition (Fig. 1.3 c).

Our second main question regarding the **mixture function** was: How herbaceous litter influences the decomposition process of tree litter, by studying its effect on litter moisture content, nutrient transfer between litter species, and through its indirect effect on soil fauna communities (Fig. 1.3 d). More specifically, we studied the effects of herb litter addition on the performance of two detritivore taxa (woodlice (Isopoda) and millipedes (Diplopoda)) and the mixture effects on litter decomposition of five types of tree litter by adding herb litter under laboratory conditions (Fig. 1.3 e). We further aimed to understand the mixture effects of herb litter on tree litter turnover under natural conditions, via known litter mixture effects underlying the decomposition in tree litter in mixtures (outlined in section 1.2): the effect on the decomposer community and the effect on nutrient transfer (Fig. 1.3 f). We have the following more specific expectations:

i) We expect that the understorey herbaceous layer will decrease soil temperature and soil moisture, and further decelerate decomposition rates of standardized litters.

ii) We expect that herb litter will have synergistic (positive) effects on litter decomposition, by increasing litter moisture, promoting decomposer’s consumption activity, and improving nutrient content of tree litter via nutrient transfer (from herb litter to tree litter).

iii) At last, we expect that the effect to be the strongest with low-quality tree litter, due to quality divergence between high-quality herb litter and low-quality tree litter.

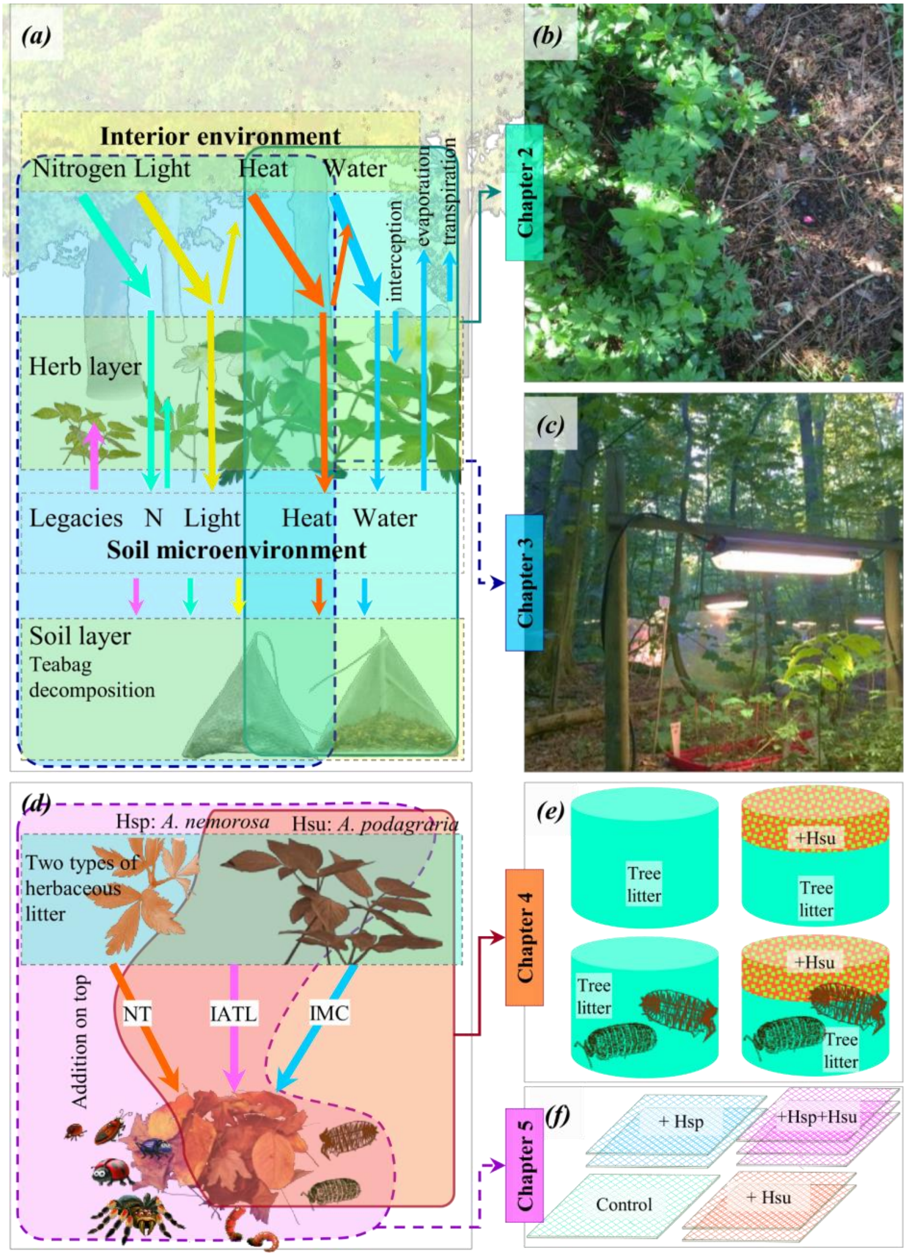


**Figure 1.3**. Schematic overview of the thesis. The mechanisms are presented left (a, d), the experiments are presented right (b, c, e, and f). (a) shows the concept of the regulatory effect of understorey herbs on the soil microclimate and litter decomposition. (d) shows the theories for litter mixture effects: improved microenvironmental conditions (IMC), Nutrient transfer (NT), and interactions across trophic levels (IATL). +Hsp: Spring herb litter addition, +Hsu: Summer herb litter addition, and +Hsp+Hsu: both spring and summer herb litter addition.

### 1.4.3 Thesis outline

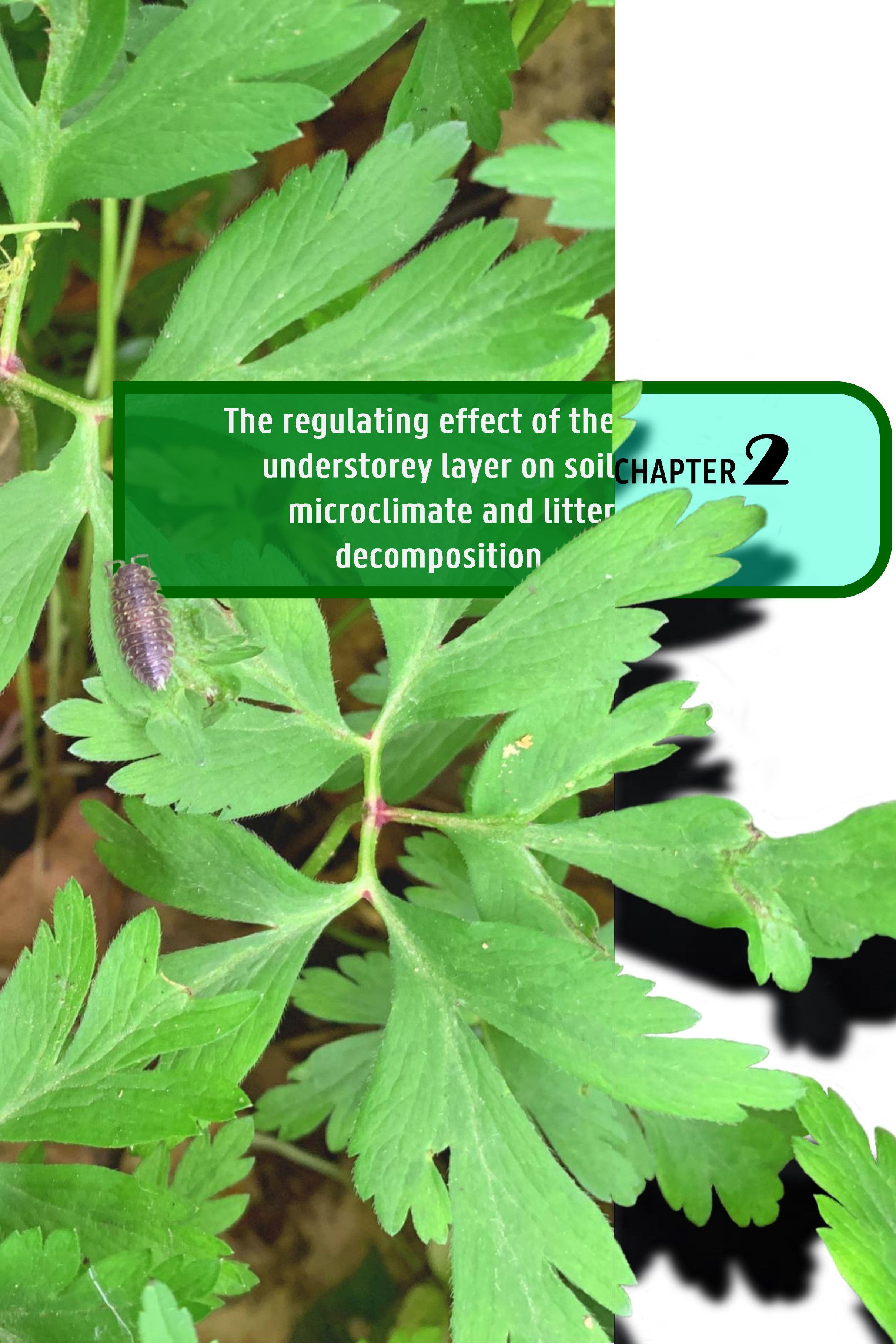
The thesis includes 6 chapters in total. In this introductory chapter (Chapter 1), the main structure and ideas of this thesis were outlined, moving from the concepts and driving factors of litter decomposition to the mechanisms of mixture effects and followed by the potential effect of the understorey layer and litter on the decomposition of tree litter.

Firstly, we aimed to understand the regulatory function of understorey herb layer on litter decomposition in temperate forest ecosystems. **Chapter 2** reports the effects of understorey herb layer on soil microclimate (temperature and soil moisture), and its effects on litter decomposition (Fig. 1.3 b). **Chapter 3** describes the regulatory function of the understorey layer on litter decomposition in the context of environmental change (Fig. 1.3 c).

Secondly, to understand the mixture effects of herb litter on decomposition of different tree litters, we conducted a mesocosm experiment under laboratory conditions and an *in-situ* litter bag experiment in a temperate mixed deciduous forest in northern Belgium. In **chapter 4**, we test the effect of herb litter addition on litter moisture, and its effects on litter mass loss and performance of detritivore during the decomposition processes, we applied *A. podagraria* litter (summer litter) and two decomposer taxa, (i.e., woodlice and millipedes) in microcosms with leaf litter of five common temperate forest species (Fig. 1.3 e). In **chapter 5**, we conducted a seasonal herb litter addition experiment that describes the mixture effects (based on theories of nutrient transfer and interactions across trophic levels) of ephemeral herb species on the decomposition of five common tree litters (Fig. 1.3 f).

Finally, in **Chapter 6**, we summarize the results, discusses the consistency and differentiate with other studies formulating suggestions for future research.

**Image:** A picture of the in-situ mesocosms in the Aelmoeseneie forests, Gontrode, Belgium, in which warming (open top chamber), enhanced light availability and nitrogen addition-treatments are applied.



# CHAPTER 2

# The regulating effect of the understorey layer on soil microclimate and litter decomposition

## Abstract①

Understorey management has a long-lasting history in silviculture, as it alleviates light, water, and nutrients competition between understorey vegetation and seedings. The soil environmental conditions are therefore different under the understorey management. However, only few studies investigated the effects of understorey removal on the soil microclimate and litter decomposition in temperate forest. In this study, we monitored soil temperature using miniature temperature sensors buried at 5 cm belowground in the presence and absence (artificial remove) of (*Anemone nemorosa* dominant) the understorey layer. We also investigated the response of volumetric soil moisture content at 0-5 cm depth and litter decomposition (by using two standardized green and rooibos tea) to the understorey removal. Our results showed that the understorey removal increased soil temperature in the beginning of the incubation, the effects decreased gradually with the incubation, as the dominant ephemeral species gradually senesced and the percentage cover of understorey vegetation gradually decreased. Soil moisture content increased in the understorey removal on both rainy and sunny days. Nonetheless, the changes in soil microclimate conditions did not affect litter moisture content and mass loss of both green tea and rooibos tea in a short-term. The percentage cover of the understorey was negatively correlated with mass loss of two types of litter. Furthermore, the correlation of soil temperature, soil moisture, and litter moisture on litter mass loss were litter identity-related. Our results suggested that the understorey layer is an important functional component in soil microclimate in temperate forests, but removal of the understorey vegetation would not immediately exert its influence on litter decomposition in soil. A longer-term study is needed to investigate the responses of microbial community and organic degradation to the structural changes in understorey and understorey litter inflow.

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① Wang, B., De Smedt, P., Verheyen, K., Baeten, L. 2020. The regulating effect of the understorey layer on soil microclimate and litter decomposition. *Forest ecology and management,* (unpublished)

## 2.1 Introduction

In forest ecosystems, overstorey tree canopy plays an essential role in the buffering of extreme climatic events, such as heat waves, heavy rains and strong winds creating a buffered microclimate below the canopy. The air temperature below the forest canopy was reported to be 1-2 °C lower than above over the forest canopy in temperate and boreal forests (Zellweger *et al.* 2020). However, before light, heat, or water reaches on the forest floor, it may be secondarily attenuated by the canopy of understorey vegetation. Simultaneously to overstorey structure, the understorey layer may play an equally important role on soil microclimate regulation, particularly in regions having a long history of forest management practices and human activities (Lorimer *et al.* 1994; Økland *et al.* 2003; Rosenberg & Jacobson 2004; Wang *et al.* 2014). Understorey management (e.g. removal or thinning) is a common technique to increase primary productivity of trees in forest management by reducing light, water and nutrient competition between understorey vegetation and tree seeding (Nambiar & Sands 1993; Pecot *et al.* 2007; Zhao *et al.* 2013). These artificial disturbances on the understorey layer are also supposed to influence the functional role of understorey layer on soil microclimate regulating. For example, some previous studies have shown that understorey herb removal could create on average a ~1°C warmer soil environment (Matsushima & Chang 2007; He *et al.* 2020). However, the understorey layer is not only important for thermal buffering, but also influences the soil moisture content via interception of precipitation, and water evaporation from the topsoil layer, where most of the fine roots of understorey species are situated (Giuggiola *et al.* 2018). The intensity of the microclimatic regulation by the herb layer and consequently further ecological processes (e.g. litter decomposition), can be related to the percentage cover of the herbaceous layer, as the understorey canopy becomes thinner, the less solar radiation is obstructed and more throughfall penetrates into the forest floor, but this effect is poorly understood.

As an essential component in carbon and nutrient cycling in forest ecosystems, litter decomposition is closely associated with the soil microclimate, in particular soil temperature and soil moisture (García-Palacios *et al.* 2013). Plant residues in a warmer or a moister soil environment generally have a faster decomposition rates compared to residues in cold or dry environments, due to the dependency of decomposers and enzymatic activity (two essential ways of litter being decomposed) on warmth and moisture (De Smedt *et al.* 2018; Wang *et al.* 2019a). Therefore, changes in below- and near-ground microclimates, caused by the differences in herb cover, can further alter decomposition processes. Only few studies have investigated the effects of an understorey removal on litter decomposition, and it generally slowed down turnover rates of forest litter in subtropical and boreal forests (Wang *et al.* 2014; De Long *et al.* 2016). The negative correlation between herb cover and litter decomposition was partly attributed to the microclimate below the herb layer, where solar radiation and rainfall only fractionally penetrate into the forest floor due to the secondary reflection of solar radiation and interception of precipitation by the understorey herb layer.

European temperate forests are characterized by a rich understorey, and wood anemone (*A. nemorosa*) is a representative understorey species with short growing season, high nitrogen content, and a high leaf area index. It is an ephemeral species with leaves sprouting from perennial rhizomes during March to April and it withers by July (De Frenne *et al.* 2010; Jagodziński *et al.* 2016; Wang *et al.* 2021). Wood anemone is therefore already present early in the growing season, forming a thick understorey canopy, hindering solar radiation, light and throughfall to reach the soil surface, thus, reducing soil warming and humidification. Consequently, litter decomposition on the forest floor could slow down. On the other hand, responding to the relative colder and dryer environment underneath the herb litter, soil fauna could migrate to a warmer and more humid place with a lower herb cover, causing differences in litter decomposition on small scales depending on the herb cover.

The main objective of this study was to investigate the effects of understorey removal on the forest floor microclimate (soil temperature, and moisture content in both the soil and the litter layer) and its further effects on litter decomposition, we conducted an understorey removal experiment and supplying with two standardized litter types (i.e. green and rooibos tea Keuskamp *et al.* 2013; Djukic *et al.* 2018). Specifically, we address the following hypotheses: (i) the herb layer functions as a buffer against temperature extremes and intercepts rainfall leading to reduced temperatures and soil moisture on the forest floor, (ii) the altering effects of the understorey layer on soil temperature and moisture depends on the percentage cover of understorey vegetation, with higher thermal and hydrological buffering with increasing understorey cover, (iii) due to the contrasting microclimatic conditions, litter decomposition will be faster when herb cover is low or absent compared to high herb cover.

## 2.2 Material and methods

### 2.2.1 Site description

The study was conducted in Aelmoeseneie forest, an ancient temperate mixed deciduous forest in Northern Belgium (East-Flanders, 50°58.5′ N, 3°48′ E, 16 m a.s.l.). The forest has a total area of 39.5 ha and the oldest trees are about 100 years old. It has a temperate Atlantic maritime climate with a mean annual precipitation of *ca.* 768 mm, rainfall is evenly distributed throughout the year. The mean annual temperature is 11.3°C, with around -0.5°C in the coldest months (January and February) and around 18.5°C in the warmest months (July and August). Beech (*Fagus sylvatica* L.), and pedunculate oak (*Quercus robur* L.) dominate the stand which is interspersed with less abundant tree species such as sycamore maple (*Acer pseudoplatanus* L.), ash (*Fraxinus excelsior* L.) and lime (*Tilia platyphyllos* Scop.). European rowan (*Sorbus aucuparia* L.), European hazelnut (*Corylus avellana* L.) and alder buckthorn (*Frangula alnus* Mill.) are commonly found in the shrub layer. The most abundant herbaceous species includes wood anemone (*Anemone nemorosa* L.), ground elder (*Aegopodium podagraria* L.) and lesser celandine (*Ficaria verna* Huds.). Soils in this forest are Podzols and Histosols (World Reference Base (WRB) for soil resources), which have a typical thin quaternary layer of sandy loam with a spotted texture B-horizon on a shallow impermeable clay and sand complex of tertiary origin. The humus layer is of mull- and moder- type (Wang *et al.* 2019a).

### 2.2.2 Experimental set-up

The study was carried out in 16 plots (5 × 5 m) with a high cover of *A. nemorosa* in the Aelmoeseneie forest. In early April 2019, five subplots (0.7 × 0.7 m: 0.49 m²) were randomly placed within each plot resulting in a total of 80 subplots (Fig. 2.1). The herb layer within one half of these subplots was harvested, identified to species level, and oven dried (70 °C for 48 h) for biomass measurement (Fig. A2.1). The cover (Fig. 2.1 d and h) of the tree canopy and the herb layer was measured using the *Easy leaf area canopy* application (Easlon & Bloom 2014) before the harvesting of the understorey herb layer. Next to each subplot (15 cm next to the edge), we collected litter in a wooden frame of 0.2 m× 0.2 m (0.04 m²) for litter investigation, all non-woody (leaves) and woody (branches, twigs, seeds, and husks) residues were collected, dried (at 65°C until constant weight) and weighted, the weight before and after drying was used to calculate the litter biomass and moisture content (Fig. A2.2). Additionally, after litter collection, a soil sample of the surface layer (0-5 cm) was collected using cylindrical rings (length 5.1 cm, Ø 5.0 cm).

The soil cores were mixed at plot level, dried, weighted and sieved through 1 mm mesh sieve. A subsample was taken for chemical analysis. The soil $pH_{(H2O)}$ was analysed with a pH-meter (Orion 920A with pH electrode model Ross sure-flow 8172 BNWP, Thermo Scientific Orion, USA) after shaking a 1:5 ratio soil/$H_2O$ mixture for 5 min at 300 rpm. For the total carbon (TC) and total nitrogen (TN) content, the soil samples were combusted at 1150 °C and the gases were measured by a thermal conductivity detector in a CNS elemental analyzer (vario Macro Cube, Elementar, Germany). The total phosphorus (TP), potassium (TK), calcium (TCa), and magnesium (TMg) were measured by atomic absorption spectrophotometry (AA240FS, Fast Sequential AAS) after complete destruction of the soil samples with $HClO_4$ (65%), $HNO_3$ (70%) and $H_2SO_4$ (98%) in teflon bombs for 4 h at 150°C (Fig. 2.2, Table A2.1).

Temperature was monitored with a pair of miniature temperature sensors (type HOBO 8K Pendant Temperature/Alarm Data Logger – UA-001–08) randomly installed at 5 cm deep in the soil (the same depth as at which the tea bags were buried) in one of five subplots per plot (Fig. A2.3, Orange point), one sensor was buried in the intact herb layer side, the other in the harvested side. In total, 15 pairs of temperature sensors were buried, however 5 pairs of sensors could not be used because their battery levels dies (3) or they were placed in plots with a low (<75%) canopy cover (2). Volumetric soil moisture content was measured with three replications during a sunny and a rainy (light rain with precipitation 12.4 mm) day by using a ML3 ThetaProbe (Delta-T Devices, Cambridge, United Kingdom).

### 2.2.3 Litter decomposition

We used standard Lipton green tea (EAN no.: 8722700055525) and rooibos tea (EAN no.: 8722700188438) (as used by Keuskamp et al. (2013)) to assess the effect of herb layer on litter decomposition. The triangular pyramid shaped bags are made of woven nylon, with a mesh size of 0.25 mm (this mesh size excludes macrofauna, but allows access of microorganisms) and filled with 1.61 ± 0.07 g and 1.75 ± 0.03 g of green tea and rooibos tea, respectively. The green tea represents a labile plant residue with low C:N ratio and high nutrient concentration, while rooibos tea represents recalcitrant plant residues. At each of the subplots, two pair of green and rooibos teabags were buried at 2-5 cm depth in the topsoil with or without the understorey plants on the 11th of April 2019, and the teabags were left in the soil over a 90-day incubation period (till 10th of July). This results in a total of 320 teabags (16 plots × 5 subplots × 2 treatments (with or without herb cover) × 2 types of tea) buried in the field. At the end of the incubation, all teabags were collected from the forest, and the remaining tea were dried at 65°C until constant weight, the weight difference before and after drying was used to calculate tea moisture content.

The remaining material was weighed after the removal of ingrown roots. Then, combusted at 550°C for 4-6 h and re-weighed to assess the remaining ash (Wang *et al.* 2019a).

**Figure 2.1** Experiment setup and herb layer cover. Half of the understorey plants framed in 70 × 70 cm plots are harvested (a, c, and g). The percentage cover of the understorey layer is measured using the *Easy leaf area canopy* application, the output of the percentage cover is showing in (d, c, h, and i). A pair of green tea and rooibos tea is buried at 2-5 cm depth in the soil under the presence and absence of understorey, respectively (j).

### 2.2.4 Statistical analyses

Temperature (or moisture) offset was calculated as the difference between the daily temperature (or moisture) under the understorey absence and the understorey presence treatments.

All statistical analyses were performed using R (R Core Team 2018) including package lme4 (Bates *et al.* 2015), and graphs were made with the ggplot2 package (Wickham, 2009).

We reduced the dimensionality of the soil properties using Principal Component Analysis (PCA) to derive a principal axis of variation in soil characteristics. The first principal component (representing 54.6% of total variance) can be considered the major axis of variation in soil chemistry. The second component representing 20.0% of variation in soil chemistry (Fig. 2.2). Pearson's correlation analysis was applied to evaluate the correlations between the soil characteristics and the component 1 ($PC_1$) and 2 ($PC_2$) (Table A2.2). The $PC_1$ was applied for the correlation analysis between soil properties and litter mass loss, see "Soil $PC_1$" in Fig. 2.5. To assess the effect of the understorey removal on soil microclimate and litter decomposition, differences in the temperature buffering, soil moisture content, and mass loss and moisture content of green tea and rooibos tea were determined using the independent *t-test* with an alpha level of 0.05. Pearson's correlation analyses and linear mixed-effect models were used to assessed the relationships between herb cover and belowground microclimate (soil temperature and moisture) and the relationships between mass loss of two types of tea after 3 months of decomposition, and the soil characteristics (PC1), soil moisture, moisture content of the tea, soil temperature and herb cover.

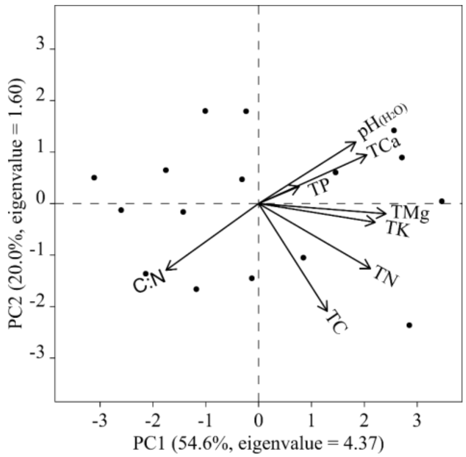

**Figure 2.2** Principal component analysis of soil properties at plot level. TC: soil total carbon, TN: soil total nitrogen, TP: total phosphorus, TK: total potassium, TCa: total calcium, TMg: total magnesium, and C:N ratio.

## 2.3 Results

### 2.3.1 Soil temperature

Across all plots, the average daily soil temperature increased from 12.52 ± 0.20°C at the beginning towards 22.58 ± 0.06°C after 90 days (Fig. 2.3a). The absence of the understorey layer significantly increased daily mean and maximum soil temperatures by 0.21 ± 0.03°C and 0.68 ± 0.08°C in first 30 days of incubation ($p < 0.001$, Table A2.3). On the contrary, in the last 30 days of incubation, the daily mean (-0.13 ± 0.04°C) and maximum (-0.42 ± 0.11°C) temperature were significant lower under the absence of understorey layer than under the presence ($p < 0.05$, Table A2.3). The understorey layer significantly buffered hot or cold thermal extremes on the crest and the trough of temperature waves, respectively (Fig. 2.3a), and led to lower daily temperature range (Fig A2.4). Across to all study plots, the understorey layer had a significant thermal offset on maximum soil temperatre, but not on mean and minimum soil temperature, reducing the temperature difference by 0.75 ± 0.08 °C between the absence and presence of understorey plants ($F_{1,\ 1790} = 100.7$, $p < 0.001$, Fig. 2.3b). In addition, the daily maximum and mean temperature offset capacity showed significant positive correlated with herb cover (Pearson's $p < 0.05$).

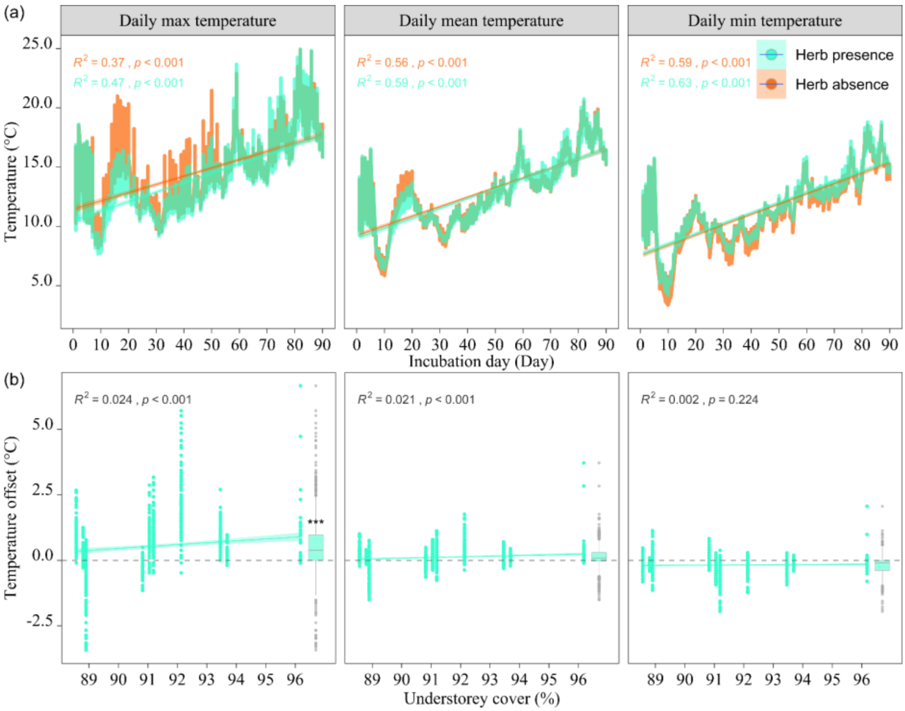

**Figure 2.3** (a) dynamic of daily maximum, mean, and minimum soil temperature during the incubation. "ns" is the abbreviation of non-significant ($p > 0.05$), and asterisks *, **, *** show significance levels with p values lower than 0.05, 0.01 and 0.001, respectively. (b) the relationship between understorey percentage cover and temperature offset (soil temperature under the understorey absence minus under the understorey presence).

### 2.3.2 Soil moisture

Soil moisture contents on sunny and rainy days were, on average, 6.81 ± 0.83% and 5.80 ± 0.93% higher under the understorey removal than under the presence of understorey ($p < 0.001$, Fig. 2.4, Table A2.4). The soil moisture offsets (soil moisture content under the understorey removal minus soil moisture content under the understorey presenting) were positively correlated with percentage cover of understorey plants, on the contrary, the moisture content showed a negative, yet non-significant, correlation with the percentage cover of the understorey layer.

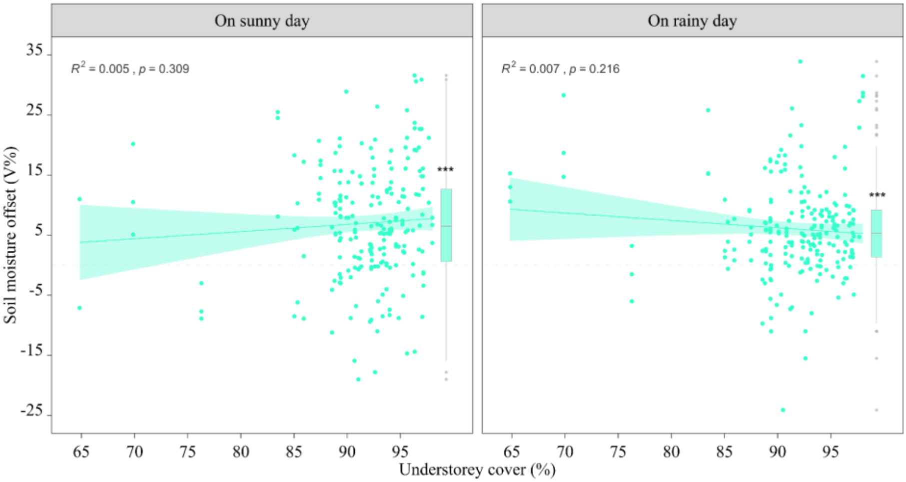

**Figure 2.4** The relationship between the understorey cover and the volumetric soil moisture offset (that is soil moisture content at understorey absence minus at understorey presence) on sunny and rainy days. The lines indicate the regression lines across the subplots with >75% overstorey canopy cover (the data were collected from 75 (out of 80) subplots). R: Pearson's correlation coefficient, *p*: Pearson's correlation *p*-value. The boxpot at the right summarized data distribution. "***" above the boxes show significance levels with *p* values lower than 0.001.

### 2.3.3 Herb layer, litter moisture and mass loss

After 90 days of incubation, mean mass loss of green and rooibos tea was 63.44 ± 0.39% and 28.52 ± 0.35%, respectively. There were no significant differences between the presence and absence of understorey plants on the mass loss and moisture content of both green and rooibos tea (Fig. 2.5). We only found the moisture content of green tea to be positively correlated with soil moisture content ($p < 0.001$, Fig. A2.4).

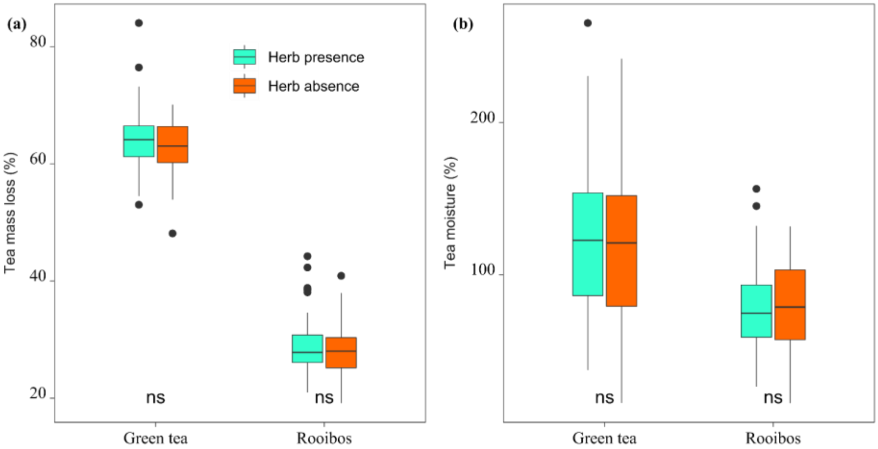

**Figure 2.5** Mass loss (a) and litter moisture content (b) of Green tea and Rooibos tea after 90 days of incubation. “ns” ($p > 0.05$), no difference in mass loss and moisture content of green tea or rooibos tea between the presence and absence of understorey layer.

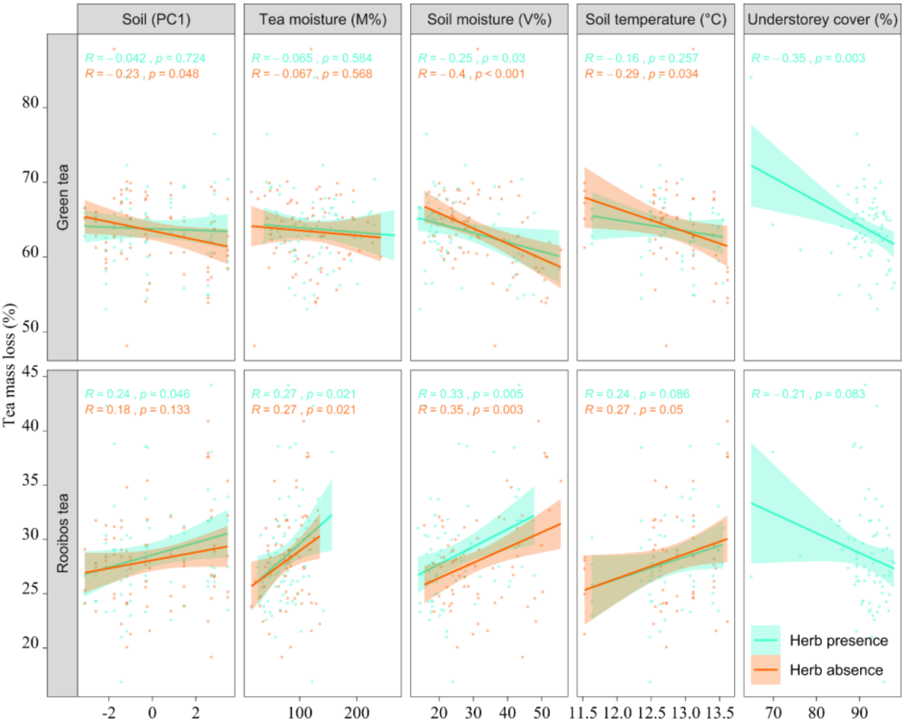

**Figure 2.6** Relationship between mass loss of two types of tea and environmental variables. The variable ‘Soil characteristics (PC1)’ indicates the component 1 of a PCA (Fig. A2.2), which is significantly related with soil C, C:N ratio, and soil nutrients (N, K, Ca and Mg) concentration (Spearman’s $\rho > 0.60$, $p < 0.05$, Table A2.5). Teabag moisture represents moisture content of the teabags (M%), while Soil moisture represent volumetric water content (V%).

### 2.3.4 Environmental variables and decomposition

No significant correlation was found between soil characteristics (PC1) and mass loss of two types of tea in understorey presenting, neither in understorey absence (Table 2.1). The mass loss of rooibos tea, but no green tea, under the presence and absence of understorey plants were positively correlated with its moisture content ($p$ < 0.05). Soil moisture content was negatively correlated with mass loss of green tea, the mass loss of green tea in more moist soil was 6.6% lower compared to in drier soil ($F_{1, 145}$ = 16.92, $p$ < 0.001), while this was opposite for rooibos tea (increase of 7.4%) ($F_{1, 143}$ = 14.51, $p$ < 0.001). Soil temperature was significantly negatively correlated with mass loss of green tea under the understorey removal ($F_{1, 71}$ = 8.54, $p$ < 0.05). There was a statistically significant, albeit weak, negative relationship between the percentage cover of understorey plants and mass loss of green ($F_{1, 70}$ = 9.79, $p$ = 0.003, Fig. 2.6), a similar, but non-significant relationship was found for the herb cover and mass loss of rooibos tea ($F_{1, 69}$ = 3.101, $p$ = 0.083, Fig. 2.6).

**Table 2.1** Effect of soil properties (PC$_1$), soil moisture, tea moisture, soil temperature, and understorey cover on mass loss of green tea and rooibos tea in understorey presence and absence treatments. Results are from linear mixed-effect models (LMM) with litter mass loss as response variable, site code as random intercept terms.

| | | Understorey presence | | | | Understorey absence | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Slope | $\chi 2$ | $p$ | | Slope | $\chi 2$ | $p$ | |
| Green tea | Soil properties PC1 | 0.598 | 1.327 | 0.249 | | -0.549 | 2.681 | 0.102 | |
| | Soil moisture | -0.046 | 0.608 | 0.436 | | -0.204 | 11.288 | **<0.001** | *** |
| | Tea moisture | 0.007 | 0.251 | 0.617 | | -0.006 | 0.281 | 0.596 | |
| | Soil temperature | 0.318 | 0.000 | 0.991 | | -2.991 | 3.842 | **0.050** | * |
| | Understorey cover | -0.376 | 15.198 | **<0.001** | *** | | | | |
| Rooibos tea | Soil properties PC1 | 0.540 | 2.482 | 0.115 | | 0.272 | 1.162 | 0.281 | |
| | Soil moisture | 0.171 | 6.911 | **0.009** | ** | 0.141 | 8.218 | **0.004** | ** |
| | Tea moisture | 0.043 | 4.913 | **0.027** | * | 0.047 | 6.934 | **0.008** | ** |
| | Soil temperature | 1.366 | 1.143 | 0.285 | | 1.953 | 2.610 | 0.106 | |

## 2.4 Discussion

We experimentally investigated the effects of the understorey layer on soil microclimate and litter decomposition in a temperate forest in northern Belgium. Our results showed that the understorey layer showed a thermal and hydrological buffering capacity. The daily maximum temperatures in the topsoil were lower when the understorey layer is presenting in field compared to the understorey removal treatment, while there was no difference for minimum temperatures. Additionally, the presence of an understorey layer causes a significant lower soil moisture level compared to plots without understorey cover on both sunny and rainy days ($p < 0.001$, Fig. 2.4 boxplot). Nonetheless, the adverse microclimatic conditions caused by the presence or absence of the understorey layer did not influence decomposition of green tea and rooibos tea, however, decomposition of the two types of tea was negatively correlated with the percentage cover of understorey layer. These results suggest that there might be a understorey-mediated trade of between soil microclimate and litter decomposition.

### 2.4.1 Effects of understorey layer on the soil microclimate

In terrestrial forest ecosystems, the understorey layer does not only play an important role in forest productivity, biodiversity conservation and nutrient cycling, but also in secondary regulation of the microclimate on the forest floor (Landuyt *et al.* 2019). Besides, the understorey herbs could have a similar function as overstorey tree species in regulating the microclimate on the forest floor via light reflection, rainfall interception, and water transpiration (Matsushima & Chang 2007; De Frenne *et al.* 2019; Zellweger *et al.* 2020).

In accordance with our first hypothesis, we observed a cooler soil temperature (daily mean and maximum temperature) and a lower soil moisture content (on rainy and sunny days) underneath the herb cover. The thermal buffering in our study (about 0.12°C and 0.56°C for daily mean and maximum temperature respectively) is much lower compared to previous research, resulting in a ~1 °C increased after herb layer removal (Matsushima & Chang 2007; Özkan & Gökbulak 2017). There are two potential explanations for the low thermal buffering in our study. A first reason might be the height and thickness of the herb layer (Vanneste *et al.* 2020). Much of the previous research was done in a grass dominated herb layers in forests, with species such as *Calamagrostis canadensis* (Matsushima & Chang 2007), *Brachypodium pinnatum* (Özkan & Gökbulak 2017) and *Imperata koenigii* (He *et al.* 2020). In our study, however, the dominating herb species is, *A. nemorosa*, a low-statured species with an average height of only 14 cm and a low stem-density (Shirreffs 1985; Lukianchuk *et al.* 2017). The species is therefore much shorter compared to most grass species in forests (Shirreffs 1985). Secondly, our experiment ran for 90 days limited by the growing season of *A. nemorosa*, therefore we possibly missed long-term effects on the microclimate induced by the presence or absence of the herb layer like effects through such soil moisture and soil bulk density (Liu *et al.* 2005; Giuggiola *et al.* 2018; Lei *et al.* 2018; He *et al.* 2020).

Wu *et al.* (2011), Zhao et al., (2013), and Wang *et al.* (2014) monitored a full year of soil temperature and soil moisture in subtropical region in China, they report a similar result being that understory removal increased soil temperature but decreased soil water content, particularly in summer. While, the understorey in that region is dominated by *Dicranopteris dichotoma*, which is a perennial-leaved species with taller height (~90 cm) compared to *A. nemorosa*. Consequently, it even led to a stronger decrease in soil temperature in summer than in spring. A two-year observation in Canadian boreal forests, in which the understorey layer is dominated by *Epilobium angustifolium*, showed that the mean summer soil temperature was increased by 0.73 °C by understory removal (Matsushima & Chang 2007). *E. angustifolium* is also a species taller (50-150 cm) than *A. nemorosa*. The regulation capacity of understorey vegetation in soil microclimate seems mainly related to a tall and dense understorey canopy cover.

The understorey layer also plays an important role in hydrological redistribution of moisture on the forest floor (Wang *et al.* 2016). On the one hand, the herb layer in forests can intercept some throughfall before it reaches the soil surface, reducing the amount of water input to the soil. In our study, the understorey layer led to 5.51±1.14% higher soil moisture compared to the understorey removal. On the other hand, the herb layer depletes water from the top soil via leaves evapotranspiration. In consequence, we observed that the soil moisture decreased faster under the presence of understorey than under the understorey removal. These two patterns cause the soil underneath the herb layer to become dryer compared to plots without understorey cover (Giuggiola *et al.* 2018) consistent with our first hypothesis and supported by our results.

The positive relationship between percentage cover of the understorey and the offset of soil temperature (daily maximum, Fig. 2.3a) and moisture (on sunny day, Fig. 2.4) in our study supports our second hypothesis. Higher understorey cover can reflect more light and solar radiation (as the most important thermal source on the forest floor) and leads to more evapotranspiration from the topsoil (especially during sunny days). This resulted in a cooler and drier soil microenvironment (Coates *et al.* 1991; Vanneste *et al.* 2020). Additionally, the difference in temperature and soil moisture between the absence and presence of an understorey layer increased with increasing herb cover, supporting the role of the understorey layer in influencing the soil microclimate.

### 2.4.2 Herb layer effects on litter decomposition

We found no support for our third hypothesis stating that litter decomposition will be faster when the understorey layer is absent. The understorey removal did not influence decomposition of both green tea and rooibos tea, despite the microclimatic differences between the presence and absence of understorey treatments. A likely explanation for the observed pattern could be that the short-term removal did not really affect the microbial decomposer communities in the topsoil. Matsushima and Chang (2007) and He *et al.* (2020) reported that there was no significant effect of understorey removal on soil microbial biomass in the early stage of incubation. However, we observed that the understorey layer (compared with the understorey removal) reduced the daily mean temperature and soil moisture as important drivers for the soil microbial community (He *et al.* 2020), but possibly the observed microclimatic changes were too small to affect the microbial community, and it did not lead to environmental stress to the microbial decomposers.

Environmental variables such as soil properties, soil temperature, soil moisture, and vegetation structure, are known to affect the rate of litter decomposition (Frøseth & Bleken 2015; Ellert *et al.* 2016; Petraglia *et al.* 2019). Our study did not detect effects of soil properties on the decomposition of both green and rooibos tea which could possibly be attributed to the scale of our study system. All studied plots are embedded in the same forest complex, the soil conditions were therefore quite similar. For example, the soil pH range was 3.7-5.7 and soil C/N ratio range was 11.1-14.4. Soil moisture is another important variable determining litter decomposition between plots (García-Palacios *et al.* 2016). We found that soil moisture was significantly correlated with mass loss of the both two types of tea, but interestingly both teas reacted differently; i.e. higher moisture values induced a positive effect on the decomposition of rooibos tea whereas there was a negative correlation with the decomposition of green tea. This could be attributed to the higher content of hydrophilic substances in green tea, allowing it to lock more water in wetter soil, whereas higher soil moisture can lead to green tea particles to aggregate together. In addition, green tea is known to be richer in tea-polyphenols (e.g., tannins, usually rather recalcitrant substances) than in rooibos tea (Iswaldi *et al.* 2011), the higher content of tannins are expected to improve aggregate stability of soil and litter (Erktan *et al.* 2017). Consequently, the aggregate structure and high water retention in green tea leads to oxygen stress for microbial decomposition (Sierra *et al.* 2017). Whereas the rooibos tea has a better air and water permeability than green tea, which results in a higher microbial decomposition and leaching loss of soluble substances (Zou *et al.* 2000). Therefore, the higher moisture content induces a stronger leaching of tea components since the rooibos leaves do not aggregate, explaining the positive correlation between moisture of rooibos tea and its mass loss.

In accordance with previous research in the same forest, e.g. Wang *et al*. (2019a), we observed a negative relationship between understorey cover and litter decomposition. The negative correlation might be because of the less favorable microclimatic conditions created by the understorey layer. As the herb canopy becomes denser, more solar radiation and rainfall is retarded to rich on the forest floor, and more soil water is evaporated through plant transpiration (Walker 2000; Vanneste *et al.* 2020). In this study, we found that the soil moisture offset on sunny day was slightly higher in the plot with denser understorey cover than in the plot with thinner cover, which implies a higher soil moisture evapotranspiration in denser understorey cover than in thinner understorey cover. Besides the water transpiration, the higher understorey cover may result in greater nutrient limitation in soil, as a higher amount of nutrients is taken up by plants. This would intensify the competition between microorganisms and plants for inorganic nutrients (Fontaine *et al.* 2003; Kuzyakov & Xu 2013). Plants would therefore release exudates in the rhizosphere shaping the microbial communities (Callaway & Ridenour 2004; Bais *et al.* 2006; Badri & Vivanco 2009). This is supported by our observation of many fine roots grown into the tea bags. The understorey cover induced environmental changes and biological competition result in a lower litter mass loss, since litter decomposition in forests is mostly driven by the climatic factors such as soil moisture and temperature and microbial decomposers (Wall *et al.* 2008; Petraglia *et al.* 2019; Wang *et al.* 2019b).

The removal of understorey vegetation has a long tradition in European silvicultural, and this management has left a long-lasting legacy in soil chemistry (Sayer 2006; Vild *et al.* 2018; Kadavý *et al.* 2019). As nutrient-rich organic matter in temperature forests, the understorey vegetation removal, firstly, reduced nutrients returning, e.g., *A. nemorosa* litter could return 24.5 kg N $ha^{-1}$ $yr^{-1}$ in our study area (aboveground biomass of *A. nemorosa* × N content of *A. nemorosa*). Secondly, without the herb canopy and its root system, water, soil organic matter and nutrient leaching is increased. Finally, the decomposition rates of litter and soil organic matter will be altered under low understorey vegetation cover.

In this study, we found that the soil microclimate is regulated by the understorey herbaceous layer, resulting in a lower soil temperature and soil moisture content under the living understorey plants than under the understorey removal. In response to the inferior microclimate conditions, we would assume a reduction in litter decomposition in plots with understorey plant compared to the understorey removal, which we did not detect. Alternatively, the understorey cover-induced competitive context was partly intensify this regulatory effect, which result in a negative correlation between litter decomposition and understorey cover. Furthermore, the innate difference between two types of litter are likely to have different responses to soil temperature and soil moisture content.  In all, our results indicate that the competitive relationship between understorey plants and microbial decomposers, in terms of understorey-induced soil microclimate, may exert a much greater effect in controlling belowground litter decomposition. We encourage more long-term experiments to assess the response mechanisms of biotic and abiotic processes on the microclimate changes caused by herb layer characteristics.

## Acknowledgments

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## Appendix

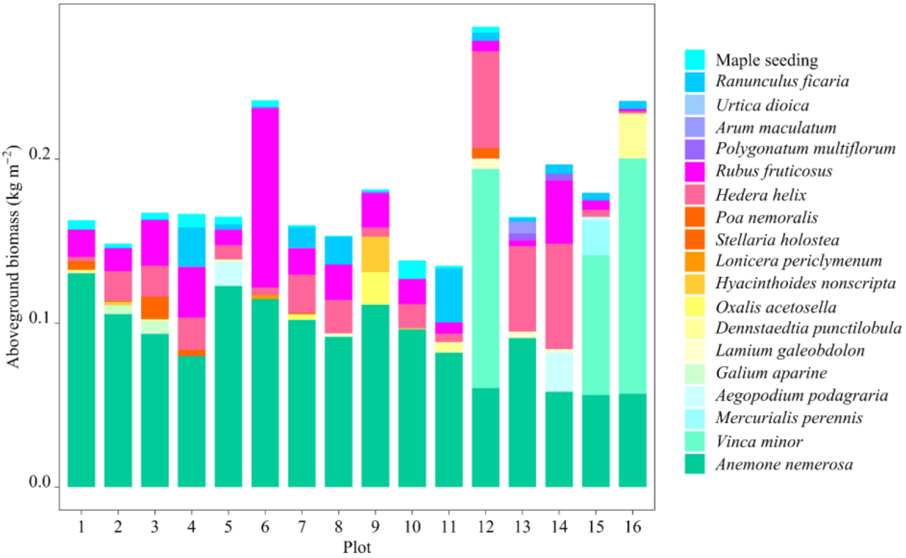


**Figure A2.1** Aboveground biomass of understorey vegetation (kg m$^{-2}$) in each plot (n = 5) in Aelmoeseneie forest.

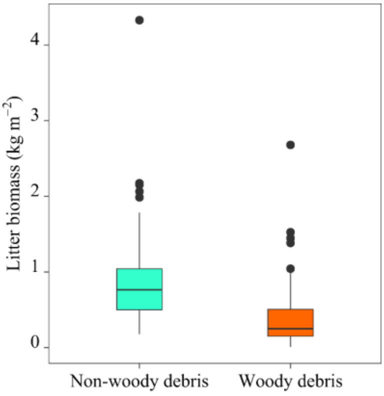


**Figure A2.2** Biomass of non-woody and woody debris (kg m$^{-2}$) in each plot (n = 5) in Aelmoeseneie forest.

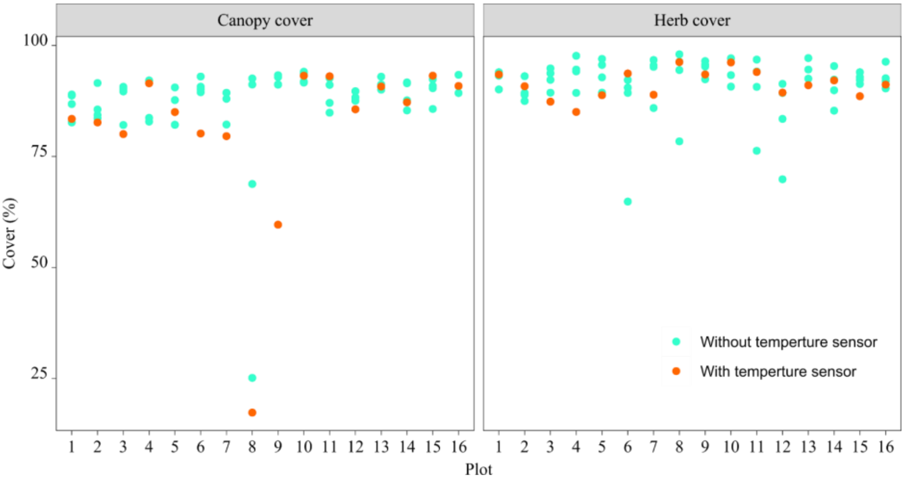


**Figure A2.3** Canopy and herb layer cover in the 16 plots. (Orange point represent where the temperature sensors were installed).

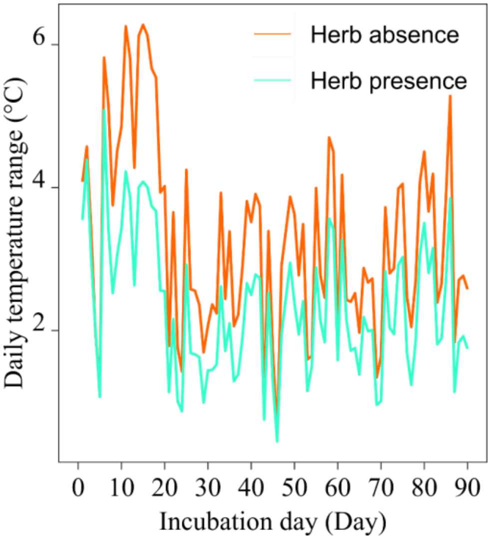


**Figure A2.4** The range of daily soil temperature (daily maximum – minimum soil temperature) under the understorey herb presence and absence treatments.

**Table A2.1** Soil physicochemical properties in 16 plots in Aalmoeseneie forest.

| Plot | Soil bulk density (g cm$^{-3}$) | pH $_{(H_2O)}$ | TC (%) | TN (%) | TP (mg kg$^{-1}$) | TK (mg kg$^{-1}$) | TCa (mg kg$^{-1}$) | TMg (mg kg$^{-1}$) | C:N |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.89 | 3.88 | 5.19 | 0.43 | 450.50 | 3591.51 | 1175.68 | 1765.24 | 11.99 |
| 2 | 0.92 | 4.00 | 3.72 | 0.33 | 866.42 | 2493.39 | 942.07 | 1376.94 | 11.37 |
| 3 | 0.81 | 3.97 | 6.12 | 0.52 | 609.28 | 4170.37 | 1379.31 | 2100.15 | 11.80 |
| 4 | 0.98 | 4.24 | 4.34 | 0.39 | 475.54 | 3876.99 | 1587.34 | 2070.02 | 11.12 |
| 5 | 1.06 | 3.92 | 4.72 | 0.39 | 357.95 | 2192.28 | 847.77 | 1192.49 | 12.11 |
| 6 | 0.93 | 3.83 | 5.45 | 0.40 | 256.22 | 2560.70 | 901.41 | 1363.24 | 13.49 |
| 7 | 1.11 | 3.94 | 4.11 | 0.34 | 228.51 | 2423.45 | 1000.00 | 1311.75 | 12.16 |
| 8 | 0.91 | 4.03 | 5.44 | 0.41 | 341.29 | 2882.34 | 1361.26 | 1717.61 | 13.37 |
| 9 | 1.05 | 3.67 | 3.88 | 0.29 | 297.73 | 2525.50 | 648.61 | 1026.69 | 13.55 |
| 10 | 0.95 | 3.97 | 4.39 | 0.31 | 346.84 | 2534.32 | 696.07 | 1084.50 | 14.01 |
| 11 | 0.98 | 3.78 | 5.02 | 0.35 | 231.57 | 2826.13 | 703.76 | 1206.60 | 14.43 |
| 12 | 0.81 | 5.24 | 4.71 | 0.42 | 378.97 | 4221.89 | 3100.66 | 1896.58 | 11.24 |
| 13 | 0.71 | 5.70 | 5.57 | 0.47 | 392.48 | 3733.12 | 3845.19 | 1985.57 | 11.88 |
| 14 | 0.79 | 5.65 | 4.74 | 0.42 | 370.50 | 3278.35 | 3891.02 | 1921.36 | 11.42 |
| 15 | 1.05 | 4.45 | 4.38 | 0.35 | 288.66 | 3170.13 | 1633.54 | 1626.76 | 12.60 |
| 16 | 1.04 | 4.88 | 3.94 | 0.34 | 275.44 | 2809.59 | 2206.11 | 1471.11 | 11.63 |

**Table A2.2** Pearson correlations between the soil characteristics and the component 1 ($PC_1$) and 2 ($PC_2$) of the soil properties PCA (Fig. A2.1c). Asterisks shows significance: * $p< 0.05$, ** $p < 0.01$ and *** $p < 0.001$ level.

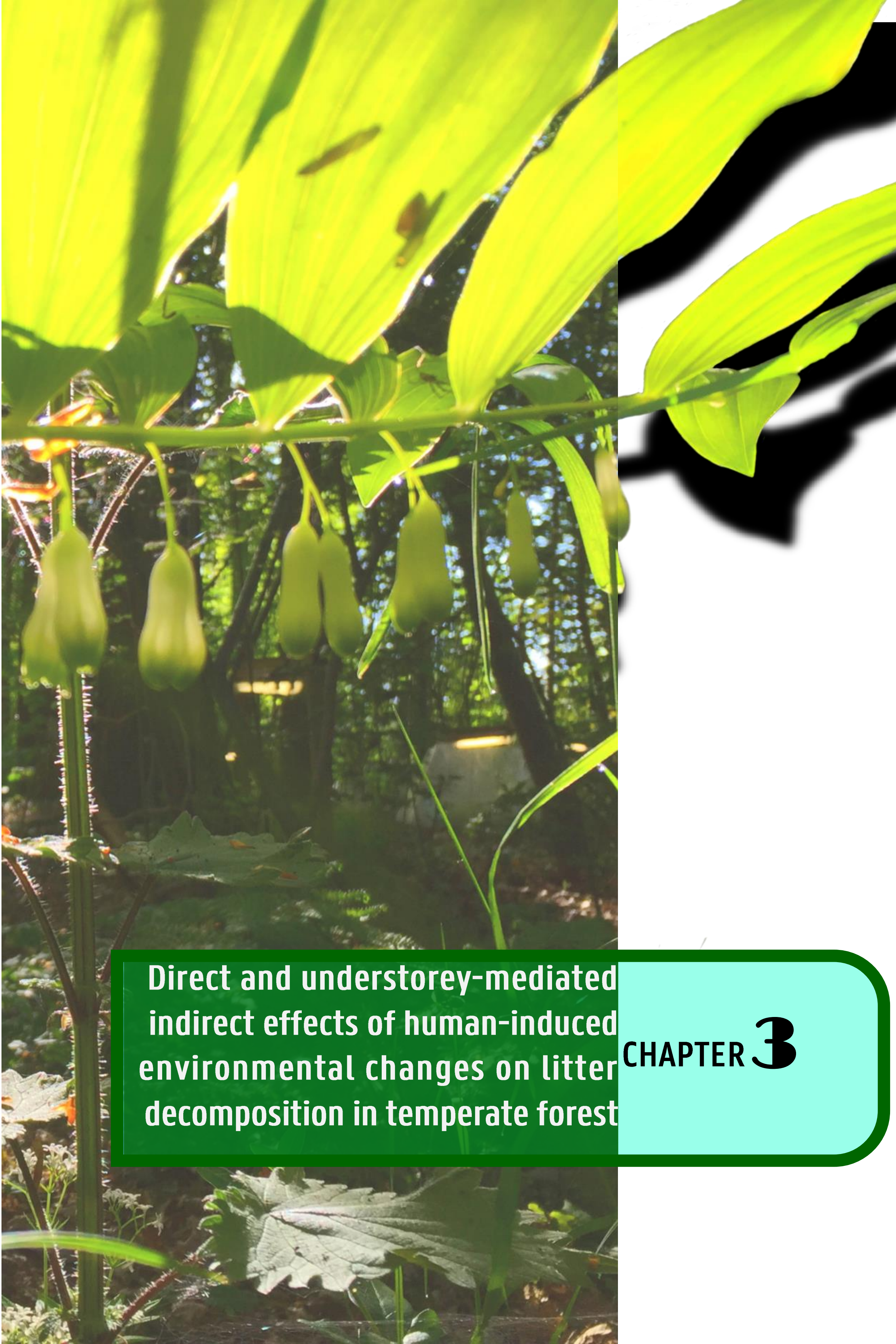
| | pH$_{(H_2O)}$ | TC | TN | TP | TK | TCa | TMg | C:N |
|---|---|---|---|---|---|---|---|---|
| PC1 | $0.73^{**}$ | $0.51^{*}$ | $0.84^{***}$ | 0.30 | $0.87^{***}$ | $0.81^{***}$ | $0.95^{***}$ | $-0.69^{**}$ |
| PC2 | $0.47^{*}$ | $-0.83^{***}$ | $-0.50^{*}$ | 0.13 | -0.14 | 0.37 | -0.07 | $-0.51^{*}$ |

**Table A2.3** Effect of herb layer removal on daily maximum ($T_{max}$), mean ($T_{mean}$), and minimum ($T_{min}$) soil temperature during different incubation periods (0-30, 30-60, 60-90, and 0-90 day (the whole incubation period)). Results are from univariate linear mixed-effect models (LMM) with temperature as response variable, the herb treatments (presence or absence) as fixed effect, and plot code and incubation day as random intercept terms. Values are model estimates ± SE with corresponding *p* values.

| Incubation period | Parameters | Herb presence | Herb absence | Temperature offset | $\chi^2$ | *p*-value | |
|---|---|---|---|---|---|---|---|
| 0 -30 day | $T_{max}$ | 12.32±0.27 | 13.19±0.25 | 0.86±0.19 | 19.77 | **< 0.001** | *** |
| | $T_{mean}$ | 10.83±0.20 | 11.05±0.18 | 0.22±0.17 | 1.68 | 0.20 | |
| | $T_{min}$ | 9.53±0.18 | 9.37±0.16 | -0.16±0.19 | 0.67 | 0.41 | |
| 30-60 day | $T_{max}$ | 12.87±0.30 | 13.32±0.29 | 0.45±0.17 | 6.83 | **0.01** | ** |
| | $T_{mean}$ | 11.84±0.14 | 11.89±0.13 | 0.05±0.15 | 0.08 | 0.77 | |
| | $T_{min}$ | 10.85±0.12 | 10.67±0.11 | -0.18±0.14 | 1.63 | 0.20 | |
| 60-90 day | $T_{max}$ | 16.87±0.33 | 17.17±0.33 | 0.30±0.17 | 3.05 | 0.08 | |
| | $T_{mean}$ | 15.64±0.15 | 15.69±0.15 | 0.05±0.14 | 0.13 | 0.71 | |
| | $T_{min}$ | 14.56±0.12 | 14.39±0.12 | -0.17±0.14 | 1.47 | 0.23 | |
| Whole period | $T_{max}$ | 13.87±0.35 | 14.41±0.34 | 0.54±0.14 | 15.60 | **< 0.001** | *** |
| 0-90 day | $T_{mean}$ | 12.61±0.25 | 12.71±0.24 | 0.10±0.13 | 0.55 | 0.46 | |
| | $T_{min}$ | 11.52±0.22 | 11.32±0.21 | -0.20±0.13 | 2.32 | 0.13 | |

**Table A2.4** Effect of herb layer removal on soil moisture content on sunny and rainy days. Results are from univariate linear mixed-effect models (LMM) with soil moisture content as response variable, the herb treatments (presence or absence) as fixed effect, and plot code as random intercept terms. Values are model estimates ± SE with corresponding *p* values.

| Parameters | Herb presence | Herb absence | Moisture offset | $\chi^2$ | *p*-value | |
|---|---|---|---|---|---|---|
| Sunny day | 28.03±3.03 | 34.85±3.05 | 6.81±0.83 | 62.993 | **< 0.001** | *** |
| Rainy day | 22.94±2.91 | 28.74±2.94 | 5.80±0.93 | 37.466 | **< 0.001** | *** |



# CHAPTER 3

# Direct and understorey-mediated indirect effects of human-induced environmental changes on litter decomposition in temperate forest

## Abstract②

Human-induced environmental changes in temperature, light availability due to forest canopy management, nitrogen deposition, and land-use legacies can alter ecosystem processes such as litter decomposition. These influences can be both direct and indirect via altering the performance of understorey vegetation. To identify the direct and indirect effects of environmental changes on litter decomposition, we performed an experiment with standardised green and rooibos teas. The experiment was conducted in a temperate mixed deciduous forest, and treatments (temperature, light, and nitrogen) were applied to mesocosms filled with ancient and post-agricultural forest soil. Both green tea and rooibos teas were more rapidly decomposed in oligotrophic soil than in eutrophic soil. The direct effects of the treatments on litter decomposition varied among the two litter types, incubation times, and soil fertility groups. Warming and agricultural legacy had a negative direct effect on the decomposition of the green tea in the high soil fertility treatment during the early decomposition stage. In contrast, agricultural legacy had a positive direct effect on the decomposition of rooibos tea. Soil enriched with nitrogen had a negative direct effect on the decomposition of green tea in mesotrophic soil in the early decomposition stage and on rooibos tea in later stage. The indirect effects of the treatments were consistently negative, as treatments (especially the temperature and light treatments in the early decomposition stage) had a positive effect on plant cover, which negatively affected litter decomposition. Our results indicate that warming, increased nitrogen deposition, and land use legacy can directly stimulate the decomposition of labile litter on more fertile soils. Furthermore, warming and increased light had stronger positive direct effects on understorey herbaceous cover, which leads to slower decomposition rates, especially in more fertile soils. Therefore, the indirect effects of environmental changes related to the understorey layer on litter decomposition can be more important than their direct effects, thus should not be overlooked.

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② Adapted from Wang, B., Blondeel, H., Baeten, L., Djukic, I., De Lombaerde, E., Verheyen, K. 2019. Direct and understorey-mediated indirect effects of human-induced environmental changes on litter decomposition in temperate forest. *Soil Biology and Biochemistry*, 138, 107579.[a]

## 3.1 Introduction

Litter decomposition is the dominant process of the carbon and nutrient cycles in forest ecosystems, which contributes to approximately 60 Pg C yr$^{-1}$ of the annual soil and atmospheric carbon input globally (Wang *et al.* 2010; Pan *et al.* 2011; Van Groenigen *et al.* 2014). There is a wealth of data showing that litter quality (e.g., the carbon to nitrogen ratio, lignin and cellulose content) determines litter decomposition rates and, ultimately, the dynamics and stocks of soil carbon (Thiessen *et al.* 2013; Fernandez *et al.* 2016). Environmental drivers play a major role in litter decomposition processes and can strongly influence litter decomposition rates. For instance, according to Parton et al. (2007), climate variables can explain up to 68% of the variability in litter decomposition rates on a global scale. Hence, changes in environmental conditions may have a tremendous impact on litter decomposition processes via both direct and indirect pathways. For instance, herbaceous biomass production was estimated to increase under increasing temperature (Lin *et al.* 2010); increasing biomass can reduce soil temperature, which may decelerate litter decomposition rates (Cornelissen *et al.* 2007).

Here, we focus on four environmental factors that are known to affect the function of temperate forest ecosystems and litter decomposition. We consider the effects of climate warming, increased light availability at the forest floor due to intensifying forest management, excess nitrogen due to deposition and fertilization, and land-use legacies in forests that were planted on former agricultural land (Hyvönen *et al.* 2007; Berg *et al.* 2010; De Frenne *et al.* 2010). Warming and agricultural legacy are considered factors that stimulate the decomposition of forest litter and soil organic matter because they generally lead to the growth of fungal hyphae and enhanced decomposer and enzyme activity (Davidson & Janssens 2006; Dang *et al.* 2009; Liiri *et al.* 2012). Conversely, high levels of nitrogen in soil generally decreases litter decomposition rates, especially for low quality litter (typically high carbon to nitrogen ratio and lignin content) because of the negative effects on decomposer (e.g., white-rot fungi, an important decomposer for lignin degradation), slow down lignin degradation, and intensifies the carbon that supports other microbial decomposers (Berg 2000; Treseder 2008; De Forest *et al.* 2014; Wu *et al.* 2019).

Changes in temperature, light, nitrogen, and land use additionally influence the biomass and composition of the herbaceous layer in forest ecosystems (Baeten *et al.* 2010; Verheyen *et al.* 2011; Perring *et al.* 2018). Numerous studies have shown that climate warming, increasing light availability, and nitrogen enrichment, alone or in combination, generally increase understorey biomass, which is highly correlated with plant cover (Bonan 2008; Maes *et al.* 2014). Plant community feedback on these environmental changes may, in turn, adjust the soil microclimate, and further alter litter decomposition (van Loon *et al.* 2014). Therefore, we may expect important indirect effects on litter decomposition via the understorey herbaceous layer cover from changes in temperature, light, nitrogen, and land-use history. Understorey removal experiments have shown that litter decomposition rates were negatively correlated with understorey cover because the activity of decomposers and enzymes were inhibited by lower soil temperature, light availability, and soil nitrogen concentrations under plant cover (Wu *et al.* 2011; Wang *et al.* 2014; De Long *et al.* 2016; Fanin *et al.* 2019). Yet, we still know remarkably little about how these environmental changes indirectly, via altering understorey vegetation cover, impacts litter decomposition.

The direct and indirect effects of these environmental changes on litter decomposition are not necessarily consistent between litter quality types (Coûteaux *et al.* 1995), decomposition stages, and soil conditions (Delgado-Baquerizo *et al.* 2015; Frøseth & Bleken 2015). The decomposition of recalcitrant litter is reported to be less sensitive to environmental conditions, such as soil moisture and nutrient availability, compared to labile litter (Liu *et al.* 2006; Austin *et al.* 2009; Christiansen *et al.* 2017). For example, the decomposition of high-quality litter (green tea) is more sensitive to the prevailing soil moisture condition and the nutrient availability compared to low-quality litter in the early stage of litter decomposition where most of the water-soluble substrates are released (Fanin *et al.* 2020). Moreover, the sensitivity of decomposition rates to environmental changes is also expected to be modulated by soil physicochemical properties (Portillo-Estrada *et al.* 2016). The direct and indirect environmental effects on litter decomposition may be stronger in nutrient-rich soils compared to nutrient-poor soils because nutrient-rich soils provide a more suitable environment (determined by nutrient availability, organic matter, pH, and soil moisture) for decomposers and enzymes. The indirect effects via plants cover on litter decomposition may be also stronger in nutrient-rich soil than in nutrient-poor soil, because the plant community may show better performance at the modification of the soil microclimate (van Loon *et al.* 2014).

The main goal of the present study was to elucidate the direct and indirect effects, related to the understorey vegetation cover, of changes in temperature, light availability, atmospheric nitrogen deposition, and land-use history on the decomposition of two types of litter in different soil types and at different decomposition stages. To this end, we added standardised litter (green tea and rooibos tea, cf. Keuskamp *et al.* 2013; Djukic *et al.* 2018) to a large-scale mesocosm experiment installed in Belgium. Understorey plant communities were grown on soils with contrasting characteristics (soil types and land use history), so that we could test for the consistency of environmental changes on decomposition in different soil contexts. We hypothesised that (i) the warming, enhanced light availability, and post-agricultural land-use treatments will have positive effects on the decomposition of both types of litter, whereas nitrogen enrichment will limit tea decomposition (especially of the green tea). (ii) The direct effects of the treatments and the indirect effects via understorey plant cover on the decomposition of labile litter will be greater than that on the recalcitrant litter types. (iii). The direct and indirect effects of the treatments will be more important in the early stage of decomposition (shorter incubation), especially for the labile litter, compared to the later stage of decomposition, since labile litter generally contents higher water-soluble substances, and most of these water-soluble substances and unshielded holocellulose decompose in the early stage of litter decomposition (Fioretto *et al.* 2005; Berg 2014). (iv) The treatment effects on litter decomposition are stronger in nutrient-rich soil compared with nutrient-poor soil.

## 3.2 Material and methods

### 3.2.1 Site description

This study was conducted in the Aelmoeseneie forest (50º58.5´ N, 3º 48´ E, 16 m a.s.l.), which is a temperate mixed deciduous forest in Northern Belgium (Flanders). This forest is considered an ancient forest, that is, it has been continuously forested since at least the oldest land-use map of 1775. The forest has a total area of 28 ha and the dominant trees are about 90 years old (De Frenne *et al.* 2010). Annual precipitation is *ca.* 850 mm and is fairly evenly distributed throughout the year. The mean annual temperature is 11.3°C, with 5.0°C in the coldest month (February) and 18.5°C in the warmest month (July and August). The most common tree species are oak (*Quercus robur*), beech (*Fagus sylvatica*) and ash (*Fraxinus excelsior*). European rowan (*Sorbus aucuparia*), European hazelnut (*Corylus avellana*) and alder buckthorn (*Frangula alnus*) are commonly found in the shrub layer. The species rich understorey community includes *Anemone nemorosa* L., *Ranunculus ficaria* L. and *Primula elatior* Hill. Soils are Dystric podzoluvisol and Dystric cambisol (FAO classification) in this forest, which has a typical thin quaternary layer of sandy loam with a spotted texture B horizon on a shallow impermeable clay and sand complex of tertiary origin. The humus layer is of a mull and moder type (Staelens *et al.* 2006).

### 3.2.2 Soil collection and analysis of properties

To understand how the environmental changes in temperature, light availability, atmospheric nitrogen deposition and land use influence decomposition of two types of litter in different decomposition stages and different soil types, an *in situ* mesocosm experiment was set up in the mixed mature temperate forest. The soils used in the mesocosms were collected from eight European regions (ranging from Central France to Southern Estonia), and from three ancient and post agricultural forest sites within each of those regions (48 sites in total, see Blondeel *et al.* 2018). Here, ancient forest is defined as forest that has been continuously present on the oldest reliable land use maps (most of them pre-dated 1850), and the forests that recovered since the wave of land abandonment in the 1950s are considered as post-agricultural (Blondeel *et al.* 2018). In each region, we selected three post-agricultural (recent) forests and three ancient forests according to the land-use maps. A topsoil (0-15 cm) sample with a surface of 70 x 100 cm was collected from each forest site (8 regions x 2 land use histories x 3 replicates). A subsample was taken from each of the 48 soils for the analysis of soil texture (% Clay, % Sand and % Silt) and soil chemical properties. Soil samples were dried and sieved through 1 mm mesh size sieve, then soil pH (in $H_2O$), total carbon (TC, %) and nitrogen (TN, %), total phosphorus (TP, mg·kg$^{-1}$) and calcium (Ca, μg·kg$^{-1}$) were determined as described by Blondeel et al. (2018). Based on soil texture and bedrock properties (Table 3.1), the soils were classified into three categorical groups using a cluster analysis and principal components analyses: *Oligotrophic soil* (Oligo, high sand content, low base saturation, and low pH), *Mesotrophic soil* (Meso, intermediate) and *Eutrophic soil* (Eu, with high clay content, high base saturation and high soil pH). These three resulting clusters were used as a categorical variable ''*Soil type*'' in the statistical analyses. See Blondeel et al. (2018) for more information.

**Table 3.1** Physicochemical properties of soils used in this study. Measurements are the average (±SE) of oligotrophic (Oligo), mesotrophic (Meso) and eutrophic (Eu) soils from ancient forest and post-agricultural forest in Europe.

| Land use | Ancient forest | | | Post-agricultural forest | | |
|---|---|---|---|---|---|---|
| Soil type | Oligo (n = 12) | Meso (n = 9) | Eu (n = 3) | Oligo (n = 11) | Meso (n = 8) | Eu (n = 5) |
| TC (%) | 4.00±0.48 $^{a}$ | 3.09±0.55 $^{a}$ | 3.78±0.96 $^{a}$ | 3.04±0.50 $^{B}$ | 2.67±0.59 $^{B}$ | 6.19±0.74 $^{A}$ |
| TN (%) | 0.25±0.03 $^{a}$ | 0.26±0.03 $^{a}$ | 0.31±0.06 $^{a}$ | 0.19±0.03 $^{B}$ | 0.24±0.03 $^{B}$ | 0.44±0.04 $^{A}$ |
| C/N ratio | 16.10±0.75 $^{a}$ | 12.00±0.87 $^{b}$ | 11.90±1.50 $^{b}$ | 14.40±0.78 $^{A}$ | 11.40±0.92 $^{A}$ | 13.90±1.16 $^{A}$ |
| TP (mg·kg$^{-1}$) | 292.00±58.9 $^{a}$ | 380.00±68.1 $^{a}$ | 468.00±117.9 $^{a}$ | 298.00±61.6 $^{B}$ | 602.00±72.2 $^{A}$ | 694.00±91.3 $^{A}$ |
| Ca (g·kg$^{-1}$) | 1.03±4.70 $^{b*}$ | 2.33±5.43 $^{ab}$ | 4.25±9.40 $^{a}$ | 0.71±4.91 $^{B}$ | 2.84±5.76 $^{B}$ | 40.90±7.28 $^{A}$ |
| pH ($H_2O$) | 4.33±0.18 $^{b}$ | 4.99±0.21 $^{b}$ | 6.28±0.36 $^{a}$ | 4.60±0.19 $^{C}$ | 5.45±0.22 $^{B}$ | 7.00±0.28 $^{A}$ |
| Clay (%) | 12.99±2.22 $^{b}$ | 18.97±2.56 $^{b}$ | 48.90±4.43 $^{a}$ | 8.85±2.31 $^{C}$ | 22.61±2.71 $^{B}$ | 40.28±3.43 $^{A}$ |
| Silt (%) | 24.40±3.43 $^{b}$ | 47.80±3.96 $^{a}$ | 43.40±6.86 $^{a}$ | 22.20±3.58 $^{B}$ | 44.30±4.20 $^{A}$ | 46.20±5.31 $^{A}$ |
| Sand (%) | 62.58±3.55 $^{a}$ | 33.29±4.09 $^{b}$ | 7.63±7.09 $^{c}$ | 69.00±3.70 $^{A}$ | 33.06±4.34 $^{B}$ | 13.52±5.49 $^{C}$ |

TC, TN, TP and Ca indicate total carbon, nitrogen, phosphorus and calcium concentrations, respectively. Different letters (lowercases for ancient forest soil and capitals for post-agricultural forest soil) indicate significant differences among soil fertility type ($P < 0.05$) and asterisks show significant differences between two land use history soils (*, **, *** indicated significance at the $P < 0.05$, $P < 0.01$ and $P < 0.001$ levels, respectively).

### 3.2.3 Experimental design

The collected soil from each of the 48 sites was sieved through 4 mm mesh size sieve (5 mm for heavy soils) for homogenization and distributed over eight mesocosms (2 temperature * 2 light * 2 nitrogen levels of treatment). Then we placed 9 L of inert river sand in the bottom of the tray for drainage, and 13 L of sieved sample was added on top. After that, a community of herb layer species, including two ancient forest species, two fast-colonizing shade tolerant species and one nitrophilous species, were randomly planted four times in the tray according to a 4 x 5 grid during the spring of 2016 (Table A3.1, A3.2 & A3.3; Fig. A3.1 & A3.2). See Appendix for more information on the communities that were used. We randomly grouped four mesocosms in a 'plot', according to their assigned treatment combination of warming, increased light and nitrogen enrichment, which results in 96 experimental plots. These plots were randomly placed in groups of four under a tree canopy (95% cover) dominated by *F. sylvatica*, *Q. robur*, *A. pseudoplatanus*, *F. excelsior* and *Larix decidua* (the light intensity and throughfall under the canopy are relatively homogeneous), and subjected to a full-factorial combination of three treatments, including two levels of warming (T), increased light availability (L), and nitrogen-addition (N). All eight treatment combinations were replicated across the forty-eight soil origins making a total of 384 mesocosms. The temperature, light and nitrogen experimental treatments are as follows:

- **T:** With or without Open Top Chamber (OTC). The air temperature or the soil temperature were expected to increase approximately 2°C by the 75 cm-wide OTC in natural conditions (De Frenne *et al.* 2015). In our experiment, we measured a significant increase ($P < 0.05$) in daily mean air temperature (at 15 cm height) of 1.13±0.36°C by the OTC between March and end of May, but insignificant increases after May 2017 (Fig. 3.1). Both soil surface temperature at 0 cm (0.39±0.36 °C) and soil temperature at 5 cm depth (0.39±0.36 °C) increased, but not significantly, during the tea bag incubation period (De Frenne, et al., 2015).
- **L:** With or without light installation. This treatment simulates the availability of light under a thin canopy of trees. A reading of ca 5-10 µmol $m^{-2}$ $s^{-1}$ PAR is expected when tree canopy is fully covered, while the cool-white fluorescent bulbs can increase the PAR up to 30 µmol $m^{-2}$ $s^{-1}$ at 75 cm height (these most likely did not increase air and soil temperature. De Frenne, et al., 2015). During the experimental period, we measured that the illumination treatment added 23.98±4.40 µmol $m^{-2}$ $s^{-1}$ PAR to the ambient light conditions (7.79±0.68 µmol $m^{-2}$ $s^{-1}$ PAR under fully closed canopy) by use of two 18 W fluorescent tubes suspended 75 cm above the ground level of each plot (Blondeel *et al.* 2019).

These lights were programmed to follow the natural photoperiod throughout the year (De Frenne, et al., 2015).

- **N:** With or without nitrogen addition. Nitrogen was enriched by adding 0.25 L of a 2.01 g/L solution of $NH_4NO_3$ (50 kg N ha$^{-1}$ yr$^{-1}$ eq.) per mesocosm and rinsing this with 0.25 L of demineralised water. This treatment was performed four times per year at the start of spring, summer, autumn and winter season, with the control mesocosms receiving 0.5 L of demineralised water (De Frenne, et al., 2015).
- **Lu:** Land use history: mesocosms were filled with either ancient forest soil or post-agricultural forest soil. The soil physicochemical properties are shown in Table 3.1.

In addition to litter decomposition (see below), we measured total vegetation cover (%), as this is tightly linked with productivity, leaf biomass and competition for light (Muukkonen *et al.* 2006). We measured total vegetation cover as the one sided projection of all leaf area in the tray with 0% being no vegetation and 100% the whole tray area covered by vegetation. We measured total cover two times during the experiment: the first week of May (4 May) and the second week of August (11 August). We used digital RGB photographs of the mesocosms taken perpendicular to the ground surface and the "Canopy Area" software tool that measures green pixels of vegetation and recalculates this into a cover percentage (Easlon & Bloom 2014).

The volumetric soil moisture content (m$^3$.m$^{-3}$) in all mesocosms (at centre and edge) was also measured by using Delta T ML3 Thetakit (Delta T, Cambridge UK) after a dry period (no rainfall for 7 days) in September 2016 and after a rainfall event in October 2016 (see Appendix B for more information). The soil moisture content was significantly higher in mesotrophic and eutrophic soil compared with oligotrophic soil. Moreover, ancient forest soil had a significantly higher soil moisture content compared with post-agricultural forest soil. The treatments of temperature, light, and nitrogen generally led to lower soil moisture content, but the differences were not significant (Table A3.4).

### 3.2.4 Tea bag litter

According to Keuskamp et al. (2013) and Djukic (2018), green tea and rooibos tea represent two different litter qualities. The leaves of Lipton green tea (EAN no.: 8 722700 055525) with low C:N ratio (12.229 ± 0.129) represents a relatively labile litter, and the Lipton rooibos tea (EAN no.: 8 722700 188438) with high C:N ratio (42.870 ± 1.841) represent a relatively recalcitrant litter, read more quality parameters in Keuskamp's *et al.* (2013). The bags are filled with 1.61±0.07 g and 1.75 ± 0.03 g of green tea and rooibos tea, respectively. The bags are made out of woven nylon, with a mesh size of 0.25 mm. This mesh excludes macrofauna, but allows access of microorganisms to enter the bags. Before burying the tea bags into the soil, five extra green teas and rooibos teas were brought back to our laboratory to determine initial ash free dry mass (AFDM). In each mesocosm, two bags of green tea and two bags of rooibos tea, in total 1536 teabags, were installed in the upper 2-5 cm of the top soil on 5 April 2017. The teabags were collected after 90 and 150 days of incubation (384 green teabags and 384 rooibos teabags each time). The teabags were oven-dried at 65°C until constant weight. Then, the remaining material was weighted and combusted at 550°C for 4-6 h and re-weighed. The remaining AFDM after the 90 or 150 days of incubation was calculated by subtracting the weight after combustion from the weight before combustion.

### 3.2.5 Statistical analyses

All statistical analyses were performed using R (R Core Team 2018), and graphs were made with the *ggplot2* package (Wickham 2016). We first tested the differences in soil physicochemical properties (TC, TN, TP, Ca, pH, clay, silt, and sand content) between the three soil types with ANOVA (Table 3.1). Analyses were done separately for ancient and post-agricultural forest sites. We then performed post hoc analyses using Tukey's honest significance test [HSD (package laercio)]. For the litter decomposition experiment, we used incubation data from 90 and 150 days for the analyses. Focusing on AFDM as the response, we used an ANOVA to test the effects of the four treatments (temperature, light, nitrogen, and land use) and the three design variables (tea type, duration of incubation, and soil type), including all two-way interactions between the treatments and design variables. The same effects (except for the tea type) were also tested on the understorey plant cover. A correlation analysis was performed to test whether the decomposition of green tea and rooibos tea was related to soil physicochemical properties.

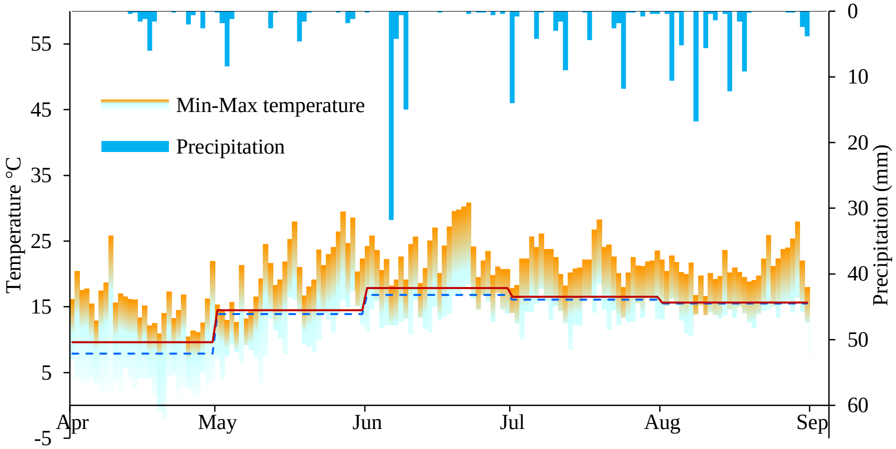

**Figure 3.1** The daily minimum and maximum air temperature (gradient filled), monthly average ambient (dashed) and warming (solid line) air temperature and daily precipitation (black filled) during the incubation period (April to September 2017) in Aelmoeseneie forest.

To understand direct and indirect relationships of the treatments (temperature, light, nitrogen and land-use history), understorey vegetation cover and litter decomposition in different litter types; and collection dates and soil fertility groups, we modelled a set of *a priori* assumed relationships (Fig. 3.2) using piecewise structural equation models (SEM). The direct effects of light on green tea and rooibos tea were not included in the SEM because the tea bags were buried in the soil, so that light addition cannot have direct effects on tea mass loss. Here, we chose to use plant cover as a proxy for understorey biomass production, which plays an important role in the interception of energy and matter (Muukkonen *et al.* 2006). The piecewiseSEM package for R was used to implement the models (Lefcheck 2016). A Fisher's *C* test was used to retain the hypothesised relationship structure (Lefcheck, 2016), the path model is considered to fit the data when the *p*-value for Fisher's *C* statistic is > 0.05 (Shipley 2009). Then standardised regression was used to calculate standardised coefficients, which were marked over the arrows. In addition, the indirect effects of the treatments (temperature, light, nitrogen and land use) were defined as the product of the standardised coefficients of the direct effects of plant cover on tea AFDM loss and the direct effect of the treatments on the cover of understorey plants.

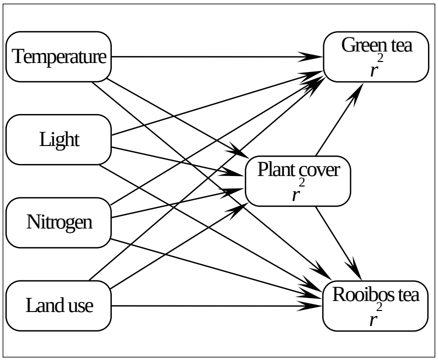

**Figure 3.2** A *priori* conceptual structural equation model depicting pathways by which temperature, light, nitrogen, land use and plant cover may influence AFDM loss of green tea and rooibos tea after 90 and 150 days of incubation in three types of soil. For each arrow, the standardised regression coefficients and overall variance explained ($r^2$) is calculated and shown in Fig. 3.5.

## 3.3 Results

### 3.3.1 Loss of tea mass

After 90 days of incubation, the AFDM of green tea and rooibos tea was significantly decreased by 64.32 ± 0.20% and 34.02 ± 0.37% of the initial AFDM content, respectively, across all treatments and soils (Table 3.2; $P < 0.001$). The AFDM of green tea and rooibos tea further significantly decreased by 4.8% and 8.3%, respectively, with an additional 60 days of incubation; therefore, the duration of incubation had a significant effect on decomposition (Table 3.2; Fig. A3.3). Also, we recorded a steadily decreasing trend of AFDM loss for green tea along the soil fertility gradients, but not for rooibos tea (Fig. 3.3). The AFDM loss of green tea was 69.17 ± 0.22%, 66.33 ± 0.27%, and 60.56 ± 0.29% of the initial mass in oligotrophic, mesotrophic, and eutrophic soil, respectively ($p < 0.05$).

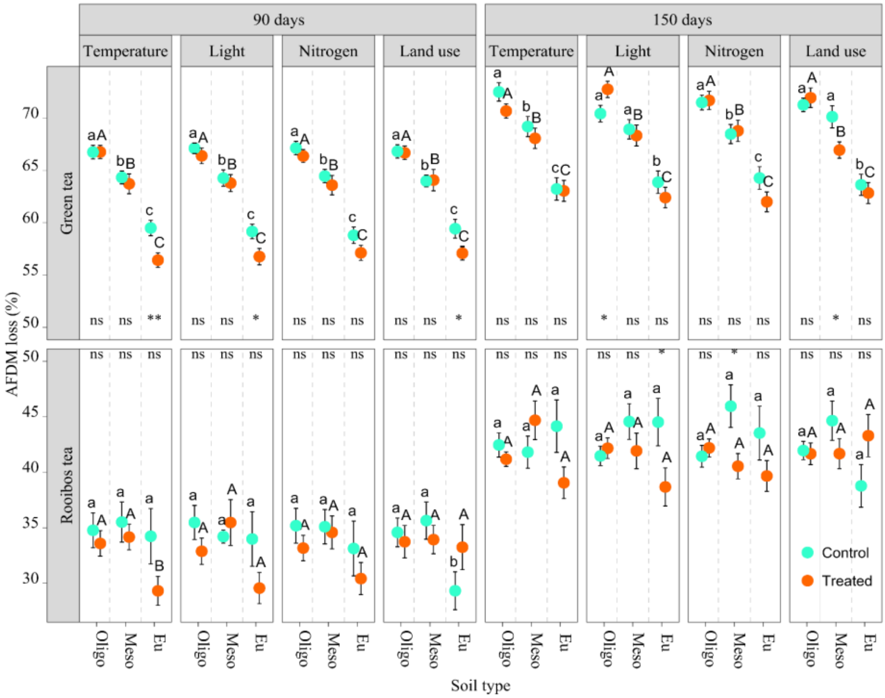

**Figure 3.3** Ash free dry mass (AFDM) loss of green tea and rooibos tea in response to four treatments applied to mesocosms: temperature, light, nitrogen and land use. Tea bags were collected after 90 days and 150 days of incubation. The experiment was performed using three soil types (oligotrophic soil (Oligo), mesotrophic soil (Meso) and eutrophic soil (Eu)). Values are means with SE. Different letters (lowercases for controls and capitals for treatments) indicate significant differences among soil types ($P < 0.05$), asterisks show significant differences between the control and treatment (ns, *, **, *** indicated significance at the $P > 0.05$, $P < 0.05$, $P < 0.01$ and $P < 0.001$ levels, respectively).

The environmental treatments (temperature, light, nitrogen, and land use) generally had a limited effect on mass loss for both tea types across soils (Table 3.2, Fig. 3.3). After 90 days of incubation on eutrophic soil, the loss of green tea mass under the treatment of warming was significantly lower (3%) than that of the control ($p < 0.05$), indicating a marginally slower decomposition with warming. As shown in Table 3.2, the interaction between soil type and treatment was significant, suggesting that the effects of temperature, light, and land-use history on the loss of mass were variable with different soil fertility groups. Additionally, there was a significant interaction between the treatment of light and tea types ($p < 0.05$). The loss of green tea mass under the increased light treatment was generally lower than the control in oligotrophic and eutrophic soil, but after 150 days of incubation, while it was significantly higher in oligotrophic soil ($p = 0.02$). Nitrogen enrichment showed a significant effect on the loss of both green tea and rooibos tea mass ($p = 0.026$); the nitrogen enrichment consistently inhibited the loss of green tea and rooibos tea mass at the two incubation times and in the three soil types (Table 3.2). Furthermore, the loss of green tea and rooibos tea mass showed limited differences among the land use treatments, except the green tea on the post-agricultural eutrophic soil had a significantly lower mass loss than that of the ancient forest soil treatment after 90 days of incubation ($p < 0.05$, Fig. 3.3).

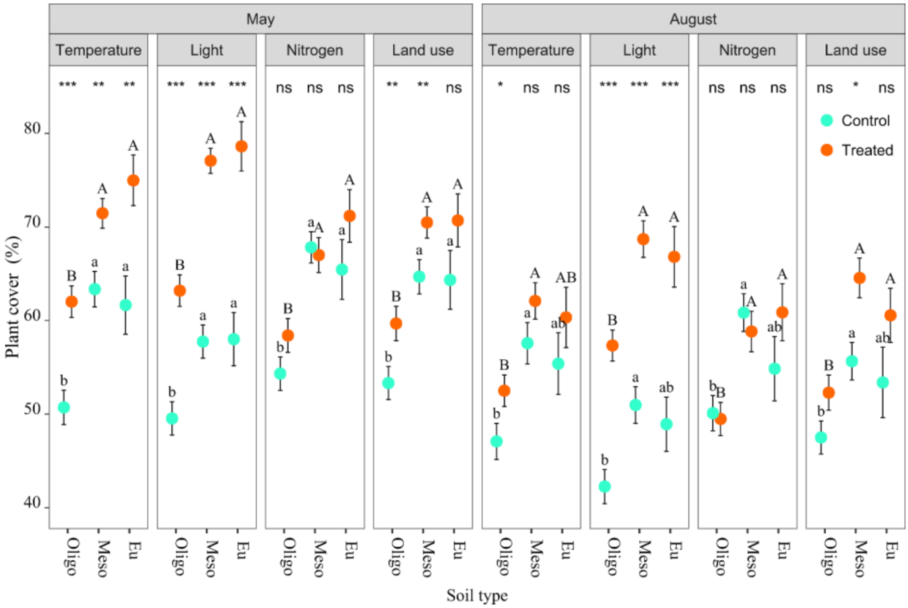

**Figure 3.4** Plant cover of mesocosm plant communities in response to four treatments: temperature, light, nitrogen and land use. Plant cover was measured 45 (spring vegetation cover, May) and 120 days (summer vegetation cover, August) after the tea bags were buried.

### 3.3.2 Effects of treatments on understorey plant cover

The average plant cover across all mesocosms was 62.27 ± 1.22% and 54.70 ± 1.29% in May and August, respectively ($p < 0.001$, Table 3.2). Soil type had a significantly positive effect on plant cover ($p < 0.001$, Table 3.2), which was approximately 10% higher in mesotrophic soil and eutrophic soil than that in oligotrophic soil. Light and land use treatments had significant positive effects on plant cover, with an increase of 16.70 ± 1.48% with increased light and increase of 6.71 ± 1.47% in post-agricultural soil (Table 3.2; Fig. 3.4). We observed a significant interaction between temperature and incubation times, with an 11% and 5% increase with warming, respectively, in May and in August. In contrast, nitrogen did not show any significant effects on understorey plant cover at the two sampling points or in the three soil types.

**Table 3.2** Effects of tea type (green tea and rooibos tea), incubation day (90 and 150 days of incubation), soil type (oligotrophic, mesotrophic and eutrophic soil), the global environmental change treatments (temperature, light, nitrogen and land use) and their two-way interaction on litter decomposition (AFDM loss) and understorey plant cover. Effects were tested with analysis of variance.

| | Litter decomposition | | | | | Plant cover | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Factor | Sum-Sq | *Df* | *F*-Value | *p* | | Sum-Sq | *Df* | *F*-Value | *p* | |
| Temperature (T) | 0.12 | 1 | 3.32 | 0.0688 | · | 23154 | 1 | 47.98 | <0.001 | *** |
| Light (L) | 0.28 | 1 | 7.74 | 0.0055 | ** | 106582 | 1 | 220.86 | <0.001 | *** |
| Nitrogen (N) | 0.18 | 1 | 4.80 | 0.0286 | * | 655 | 1 | 1.36 | 0.2441 | |
| Land use (LU) | 0.05 | 1 | 1.31 | 0.2534 | | 15534 | 1 | 32.19 | 0.0000 | *** |
| Tea type (Tea) | 135.26 | 1 | 3704.66 | <0.001 | *** | | | | | |
| Incubation (Day) | 9.04 | 1 | 247.46 | <0.001 | *** | 22041 | 1 | 45.67 | <0.001 | *** |
| Soil type (Soil) | 1.54 | 2 | 21.08 | <0.001 | *** | 39549 | 2 | 40.98 | <0.001 | *** |
| Tea: Soil | 0.48 | 2 | 6.60 | 0.0014 | ** | | | | | |
| T: Tea | <0.01 | 1 | 0.06 | 0.8089 | | | | | | |
| T: Day | 0.01 | 1 | 0.22 | 0.6367 | | 2896 | 1 | 6.00 | 0.0144 | * |
| T: Soil | 0.27 | 2 | 3.65 | 0.0263 | * | 473 | 2 | 0.49 | 0.6126 | |
| L: Tea | 0.21 | 1 | 5.68 | 0.0173 | * | | | | | |
| L: Day | 0.04 | 1 | 1.02 | 0.3130 | | 11 | 1 | 0.02 | 0.8804 | |
| L: Soil | 0.28 | 2 | 3.84 | 0.0217 | * | 1869 | 2 | 1.94 | 0.1445 | |
| N: Tea | 0.05 | 1 | 1.36 | 0.2431 | | | | | | |
| N: Day | <0.01 | 1 | 0.04 | 0.8469 | | 659 | 1 | 1.37 | 0.2428 | |
| N: Soil | 0.13 | 2 | 1.76 | 0.1729 | | 2383 | 2 | 2.47 | 0.0850 | · |
| LU: Tea | <0.01 | 1 | 0.00 | 0.9695 | | | | | | |
| LU: Days | <0.01 | 1 | 0.00 | 0.9884 | | 3 | 1 | 0.01 | 0.9334 | |
| LU: Soil | 0.27 | 2 | 3.68 | 0.0254 | * | 255 | 2 | 0.26 | 0.7681 | |
| Residuals | 55.10 | 1509 | | | | 731578 | 1516 | | | |

### 3.3.3 Direct and indirect effects

The $p$-values obtained from the Fisher's $C$ tests were >0.05, indicating that the retained relationships were a valid description of the system. The exception to this was the SEM for green tea on oligotrophic soil after 150 days of incubation. Increased light and temperature were more prominent drivers of decomposition than nitrogen enrichment and land-use legacy across soil types and incubation periods. Together, increased light and temperature explained the mainly changes in understorey plant cover ($R^2$ ranged from 0.22 to 0.60). Plant cover showed variability explaining the response of AFDM loss of green tea ($R^2$ between 0.07 and 0.42) and rooibos tea ($R^2$ between 0.06 and 0.33) across soil types and incubation periods (Fig. 3.5). The explained variation for AFDM loss of both types of tea after 150 days of incubation was substantially lower than that in 90 days of incubation. The amount of variation explained for both types of tea mass loss and plant cover showed an increasing trend from oligotrophic soil to eutrophic soil after 90 days of incubation. The temperature and light treatments had a consistently and significantly positive direct effect on plant cover in the three types of soil ($p < 0.05$). Moreover, the effect of light remained after 150 days, while the effect of temperature only persisted in mesotrophic soil.

Plant cover had a consistently and significantly negative effect on the AFDM loss of green tea and rooibos tea. The direct effects of all treatments on AFDM loss of both types of tea were limited on oligotrophic soil, while nitrogen enrichment directly inhibited AFDM loss of green tea (standardised estimate = -0.208) and rooibos tea (standardised estimate = -0.192) after 90 and 150 days of incubation on mesotrophic soil, respectively. Warming and agricultural legacy had a negative direct effect on AFDM loss of green tea, whereas agricultural legacy significantly promoted AFDM loss of rooibos tea.

When partitioning the total effects of the treatments into direct and indirect effects, we found that temperature and light represented a larger part of indirect effect across tea types, soil fertility groups and incubation times (Fig. 3.6). The indirect effects of temperature and light on green tea and rooibos tea AFDM loss showed a decreasing trend from oligotrophic soil to eutrophic soil throughout the duration of the incubation. After 150 days of incubation, the indirect effects of the treatments were generally less than that of the early stage of decomposition, with the land use treatment showing an indirect effect on the loss of rooibos tea mass on mesotrophic soil.

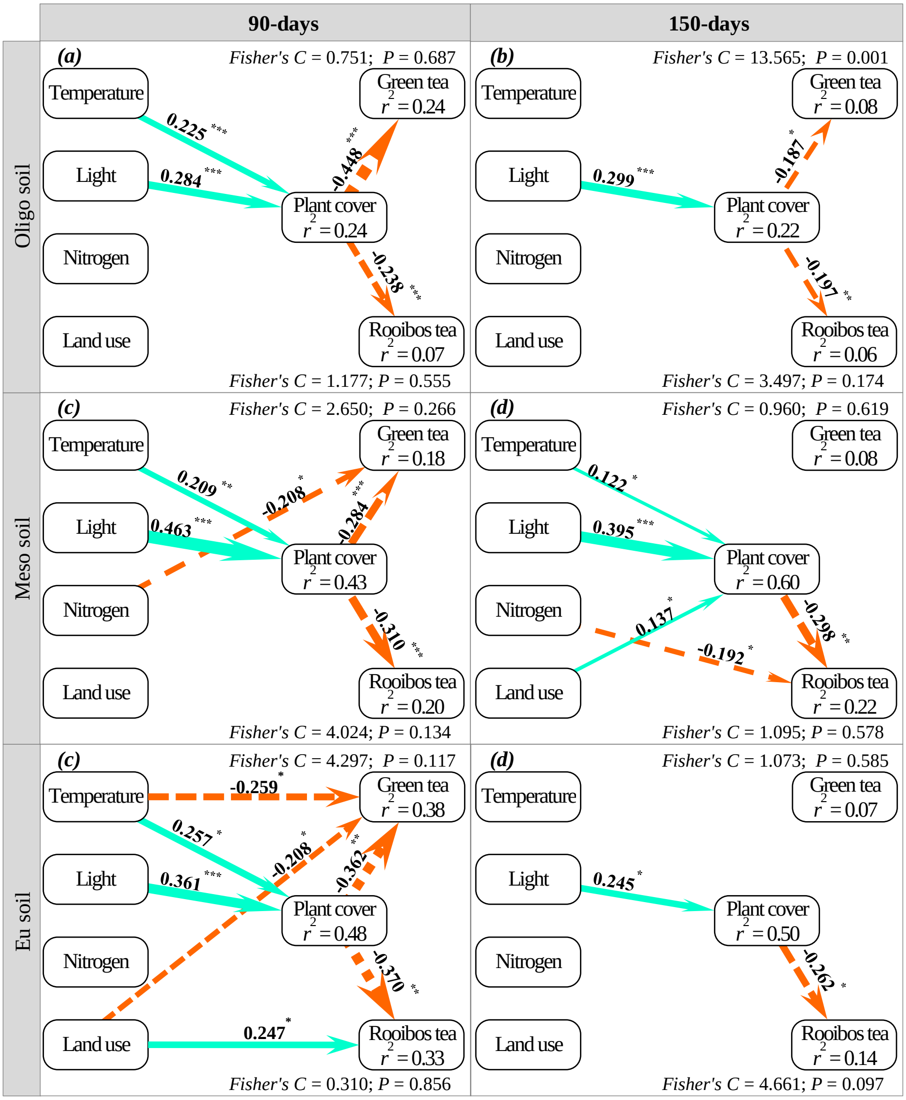

**Figure 3.5** Direct and indirect influences of temperature, light, nitrogen, land use and understorey plant cover on AFDM loss of green tea or rooibos tea after 90 (a, c, e) and 150 (b, d, f) days of incubation. Models were fitted for tea bags collected in three soil types: oligotrophic (Oligo; a, b), mesotrophic (Meso; c, d) and eutrophic (Eu; e, f) soils. The dashed and solid arrows represent the significant negative and positive effects, respectively. The number next to each arrow is the value of the standardised regression weights. Bold values are significant, and *, **, *** indicates significance at the $p < 0.05$, $p < 0.01$ and $p < 0.001$ levels, respectively. Non-significant paths were omitted from the graph, but included in the model when fit was tested.

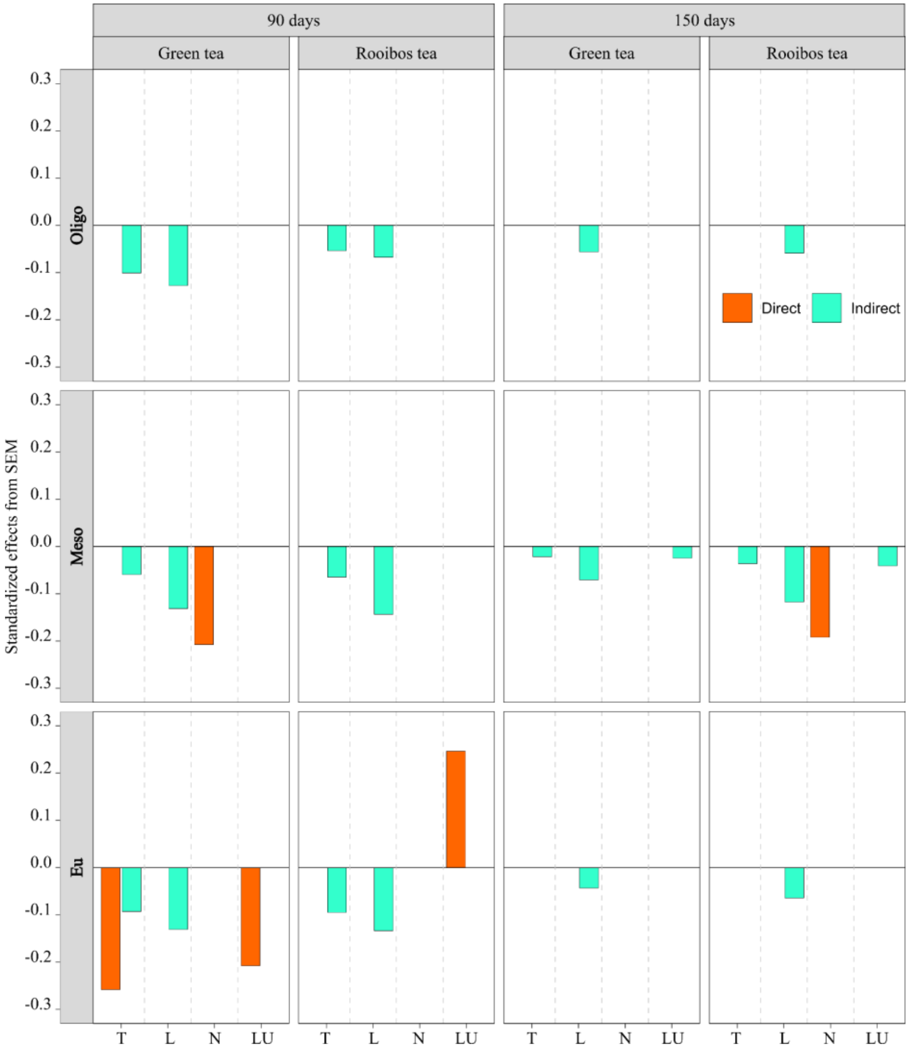

**Figure 3.6** Standardised direct effects(open) of temperature, light, nitrogen, and land use and their indirect effects (solid grey) via understorey plant cover on AFDM loss of green tea and rooibos tea in oligotrophic soil (Oligo) and mesotrophic soil (Meso) and eutrophic soil (Eu) after 90 days and 150 days of incubation. Note that the direct effects are highlighted in Fig. 3.5, the indirect effects are calculated as a product of the direct effect of treatments on plant cover and the direct effects of plant cover on tea decomposition. The omitted columns represent insignificant pathways ($p < 0.05$).

## 3.4 Discussion

Litter decomposition is generally controlled by both internal factors (such as litter quality) and external factors (such as decomposers and environmental factors; Rouifed *et al.* 2010). In this study, we explored the direct and indirect effects, via the understorey herbaceous cover, of four important human-induced environmental changes on the decomposition of labile and recalcitrant litter, represented by two tea types used as standardised litter. Inconsistent with our hypothesis, warming, increased light, and agricultural legacy did not have the expected positive effects on the decomposition of the two litter types, but consistent with our first hypothesis, we did find a reduction in decomposition with nitrogen addition. Stronger direct and indirect effects were generally observed during the early-stage of decomposition of the labile litter than on the recalcitrant litter, especially in the nutrient-rich soil, which is consistent with the second and third hypotheses. Moreover, the direct and indirect effects of the treatments were considerably stronger in nutrient-rich soil compared with nutrient-poor soil. The understorey plant cover increased with warming, increased light availability, and on post-agricultural forest soils. Since the understorey plant cover had consistently negative effects on the decomposition of both types of litter, this shows that global environmental changes may have important indirect effects on litter decomposition via the response of the understorey community.

### 3.4.1 Response of litter decomposition and plant cover to the treatments

Consistent with previous researches (Didion *et al.* 2016; Djukic *et al.* 2018; Petraglia *et al.* 2019), we found that the loss of green tea mass was approximately twice as fast as that of rooibos tea, and was more strongly influenced by the treatments than the rooibos tea (Table 3.2). This is probably due to the fact that the green tea has higher concentrations of soluble compounds than rooibos tea (Fierer *et al.* 2005), which increases the decomposition rates of green tea through leaching and the activity of microorganisms; hence, making green tea more reactive to environmental changes (Djukic *et al.* 2018). Rooibos tea is possibly composed of more stable plant matter, which remained unaffected during this short observation period (i.e., the vegetation period from April to September). This, in turn, implies that this short-term study could not capture sufficient information related to this recalcitrant material. Surprisingly, we observed a negative correlation between soil fertility groups and the loss of green tea mass, which was not displayed in the rooibos tea (Fig. 3.2). First, this can be partly attributed to the negative effect of fine mineral particles on litter decomposition in clay soils (Sollins *et al.* 1996), as it helps the litter organic components to become water-stable soil aggregates (Angst *et al.* 2017). The ash content of green tea and rooibos tea increased, on average, 0.09 g and 0.21 g, respectively. And the increase of ash content was related to soil type, the remaining ash of green tea (0.11 g) and rooibos tea (0.23 g) increased more in mesotrophic and eutrophic soil. Second, the oxygen permeability in eutrophic soil might be decreased by the high moisture content, the microbial activity therefore might be inhibited. Third, the release and leaching of elements and smaller debris particles are easily lost through the pores of sandy (oligotrophic) soils, especially at the early-stages of litter decomposition, when water soluble substances are primarily lost (Berg 2014). This may also explain why the loss of rooibos tea mass (which has very low content of water-soluble substances) was not different in sandy (oligotrophic) soil or clay (eutrophic) soil (Fig. A3.3).

In agreement with previous studies, we found that nitrogen enrichment could generally reduce decomposition rates though slowing microbial activities in soil (Treseder 2008; Janssens *et al.* 2010; Huang *et al.* 2011). However, we did not expect that warming, increased light availability, and agricultural legacy would have inhibitory effects on litter decomposition at the two incubation stages and in the three types of soil. These findings are inconsistent with previous studies that have shown that litter decomposition is stimulated by warming, increased light availability, and land-use legacy due to increases of enzyme and soil microbial activity (Fierer *et al.* 2005; Liiri *et al.* 2012). A possible explanation for these contrasting results is that the open top chambers had very limited warming effects on soil temperatures after the leaf-flushing period of overstorey trees. In our study, the chambers only successfully increased the air temperatures between 1–1.5°C before the leaves opened on overstorey trees, which can be important to understorey development (De Frenne et al 2010), and thus also for decomposition rates. When the canopy closes in late spring and summer, solar radiation is almost completely intercepted by the canopy, so the effects of warming became weaker. Thus, warming during the incubation period had a large effect on the understorey, but not on soil temperatures. Moreover, litter degradation generally has lower sensitivity to environmental change when the mean annual temperature is lower than 10°C (Prescott 2010). The average air temperature at our study site was approximately 10°C during the incubation period, and we were only able to increase the mean air temperature by 1°C with passive warming. Moreover, the soil temperature was much lower than the air temperature, which might have led to an opposite effect (slower rate of decomposition) on litter decomposition. Saura-Mas et al. (2012), Almagro et al., (2015), and Petraglia et al., (2019) also observed that warming inhibited litter decay when the mean annual air temperature was increased by less than 3°C.

Understorey plant cover showed a positive response to the treatments. Temperature, light, and land-use significantly increased plant cover by 7% to 17% during the incubation period. This is in line with the results from De Frenne et al. (2015), indicating that understorey plants have stronger responses to warming and increased light availability compared with nitrogen enrichment. Moreover, plant cover increased (especially for the understorey plants in the temperature and light treatments in May) with the increase of soil fertility. This indicates that the understorey plants exploit the additional warmth and light when the soil can supply sufficient nutrients (for example, in eutrophic soil and with agricultural legacy). The understorey plant communities growing in nutrient-rich soil may show a stronger response to warming and increased light availability than plants growing in nutrient-poor soil, especially during the growing season.

### 3.4.2 Direct and indirect effects of environmental changes

The indirect effects of environmental changes, via understorey plant cover, were calculated by multiplying the standardised direct effects of the treatments on understorey plant cover by the direct effects of plant cover on litter decomposition (García-Palacios *et al.* 2013). The indirect effects were most apparent for temperature and light and were the strongest in the early stage of decomposition. Increased plant cover is expected to slow down the soil temperature increase and reduces soil moisture content, due to sunlight shading, rainfall interception, and soil water evapotranspiration (Wahren *et al.* 2005; Niinemets 2010; Myers-Smith *et al.* 2011; van Loon *et al.* 2014). Consequently, the higher plant cover might foster a less favourable soil environment (such as maintaining a lower temperature, light, and soil nutrients) for decomposers and enzymes (De Long *et al.* 2016; Lei *et al.* 2018).

The stronger (in)direct treatment effects on the decomposition of labile litter compared with recalcitrant litter are likely to be related to differences in litter chemistry. The higher content of water-soluble substances and cellulose/hemicellulose released in the early-stage of decomposition probably led to a higher sensitivity to environmental changes in the labile litter compared to the recalcitrant litter (Portillo-Estrada *et al.* 2016). The higher absolute value of direct and indirect effects of the treatments on rooibos tea compared to green tea after 150 days of incubation also supports this, since the rooibos tea might have higher concentrations of easily decomposing substrates in the later stage of litter decomposition. In this study, the direct effects of agricultural legacies were opposite for green tea and rooibos tea in three types of soil; the effects were also observed in eutrophic soil in the later stages of the incubation period (Fig. 3.6). We found a significantly negative direct effect of land use on the decomposition of green tea on eutrophic soil, while the reverse was true for rooibos tea (Fig. 3.5). The higher concentrations of phosphorus, which generally promotes microbial degradation in systems that are less N-limited (i.e. in the post-agricultural forest soils and the eutrophic soils) likely led to higher decomposition rates of recalcitrant litter (De Long, et al., 2016). Because higher concentrations of phosphorus may have also stimulated decomposition of the recalcitrant carbon substances by increasing microbial abundance and stimulating enzyme activities (Luo *et al.* 2019).

Significant direct and indirect effects of the treatments were primarily observed in the early-stage of decomposition compared to the later stage of decomposition. This may have been due to the response of decomposers and enzymes to the changes in temperature and agricultural legacies in the early stage of incubation, which plays a dominant role during the decaying and leaching of most of labile and soluble substances (Berg *et al.* 2010). However, this pattern was not found for the nitrogen treatment. This is likely because nitrogen enrichment mainly affected the decomposition of tea in mesotrophic soil, and the effects of nitrogen enrichment were also related to the tea type and incubation time (Fig. 3.6). Knorr et al. (2005) reported that nitrogen enrichment could inhibit litter decomposition when the litter quality was low. Here, we also found that nitrogen enrichment had a direct negative effect on recalcitrant litter in the later stage of decomposition. Similarly, decomposition of green tea in early stage of decomposition was also inhibited by nitrogen enrichment. It is possible that this is due to the rapid decomposition of labile substances at the beginning (about 30 days) of the incubation period; thus, the additional nitrogen could slow the decomposition of the remaining recalcitrant components.

We found that warming, nitrogen enrichment, and agricultural legacy had stronger inhibitory effects on decomposition in more fertile soil (eutrophic soil) than that in low fertility soil (oligotrophic soil). These results are inconsistent with previous studies which have shown that litter decomposition is generally positively influenced by soil nutrient status (Vesterdal 1999; Sariyildiz & Anderson 2003). The higher temperature, soil moisture and nutrient availability in eutrophic soil might provide a suitable growing environment for fungi and plant roots (which were difficult to completely remove from the bags during sampling). In contrast, in oligotrophic soil, physical losses of organic compounds from leaching and other processes might have dominated the loss of tea mass due to the porous structure in these soils. On the other hand, the drier environment also hosted fewer growth of fungi and plant roots. The indirect effects of increased temperature and light were similar in the three types of soil (Fig. 3.6). A possible explanation is that the increased temperature and light led to a higher nitrogen uptake of plants, because of competition for nitrogen, which might intensify the activity of nitrogen-limited soil microbes in nutrient-poor soil (De

Long, et al., 2016). Consequently, the positive direct effect of vegetation cover on loss of tea mass were stronger in oligotrophic soil than in eutrophic soil, even though the direct effects of temperature and light on plant cover were weaker in oligotrophic soil than in eutrophic soil (Fig. 3.5).

In summary, our results provided evidence that human-induced environmental changes may have important direct effects on litter decomposition, especially for labile litter. However, the nature of these effects are impacted by the responses of the understorey plant community to the same environmental drivers, which are, in turn, mediated by inherent soil conditions such as soil fertility and texture. Furthermore, as our short-term experimental results imply, the decomposition rates of labile and recalcitrant litter differ strongly in the early stages of decomposition. This difference in decomposition can be accelerated by the presence of an understorey. Therefore, to further unravel the mechanisms that underlie the direct and indirect effects on litter decomposition in multiple global change contexts, additional research should be conducted on the soil microclimate.

## Acknowledgments

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# Appendix

The vegetation pool consists of fifteen common understorey species (Table A3.1), which included six ancient forest species (group 1), six fast-colonizing shade tolerant species (group 2) and three nitrophilous species (group 3). The grouping of these plants was done in emergent groups linked to colonizing capacity, as described by De Frenne *et al.* (2010). We randomly selected twelve communities from 675 possible combinations. Each community (Table A3.2) consists of five species (we randomly picked out two species from group 1 and group 2 and one species from group 3), and was replicated four times (Table A3.3). The species in each community were planted four times (twenty individuals in total) per tray at equal distances (a grid of 7 x 8,5 cm).

**Table A3.1** The pool of the fifteen selected species, which are all common understorey species in temperate European forests.

| Characteristic group | Species | Code |
|---|---|---|
| Ancient forest species (Group 1) | Wood anemone (*Anemone nemorosa*) | Anenem |
| | Solomon’s seal (*Polygonatum multiflorum*) | Polmul |
| | Lesser periwinkle (*Vinca minor*) | Vinmin |
| | Bluebell (*Hyacinthoides non-scripta*) | Hyanon |
| | Sweetscented bedstraw (*Galium odoratum*) | Galodo |
| | Wood-sedge (*Carex sylvatica*) | Carsyl |
| Fast colonizing shade tolerant species (Group 2) | Blue bugle (*Ajuga reptans*) | Ajurep |
| | Ground-ivy (*Glechoma hederacea*) | Gleched |
| | Common ivy (*Hedera helix*) | Hedhel |
| | Lesser salandine (*Ranunculus ficaria*) | Ranfic |
| | Wood bluegrass (*Poa nemoralis*) | Poanem |
| | Herb-robert (*Geranium robertanium*) | Gerrob |
| Nitrophilous species (Group 3) | Rough bluegrass (*Poa trivialis*) | Poatriv |
| | Common nettle (*Urtica dioica*) | Urtdio |
| | Ground elder (*Aegopodium podagraria*) | Aegpod |

**Table A3.2** The twelve plant communities consisting of five species each.

| **Community** | **Groep1A** | **Groep1B** | **Groep2A** | **Groep2B** | **Groep3** |
|---|---|---|---|---|---|
| Com1 | Galodo | Polmul | Ajurep | Hedhel | Poatriv |
| Com2 | Carsyl | Hyanon | Gleched | Ajurep | Aegpod |
| Com3 | Hyanon | Anenem | Poanem | Gleched | Aegpod |
| Com4 | Carsyl | Polmul | Ajurep | Ranfic | Poatriv |
| Com5 | Carsyl | Polmul | Gerrob | Hedhel | Aegpod |
| Com6 | Anenem | Vinmin | Poanem | Gerrob | Poatriv |
| Com7 | Hyanon | Carsyl | Hedhel | Gleched | Urtdio |
| Com8 | Vinmin | Galodo | Ranfic | Ajurep | Aegpod |
| Com9 | Anenem | Hyanon | Gerrob | Poanem | Poatriv |
| Com10 | Polmul | Vinmin | Gerrob | Ranfic | Urtdio |
| Com11 | Vinmin | Galodo | Poanem | Hedhel | Urtdio |
| Com12 | Anenem | Galodo | Gleched | Ranfic | Urtdio |

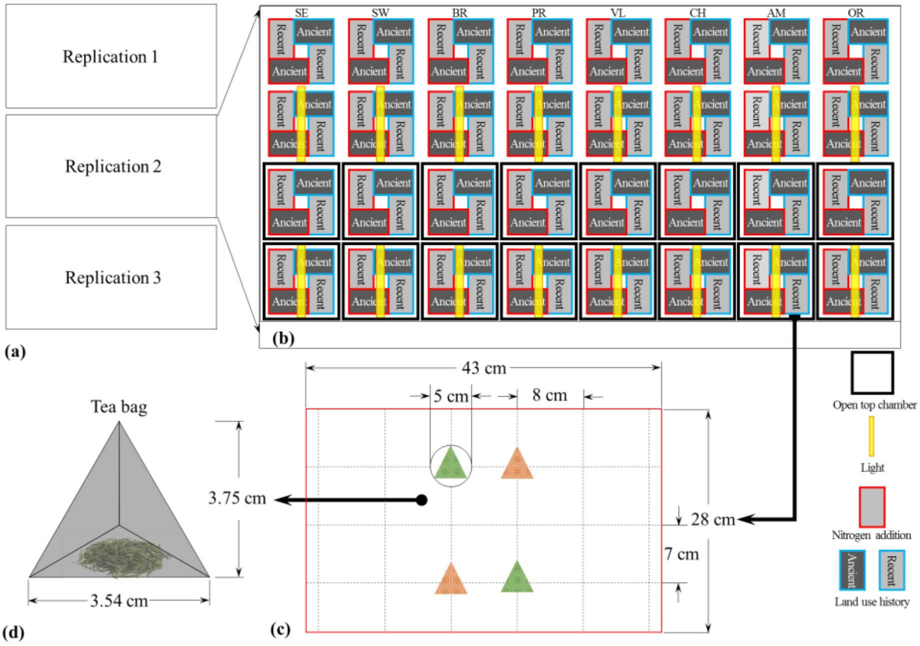

**Figure A3.1**. Scheme of the experimental design. Note: the replications (a) and the eight soil sites (b) were randomly distributed in the forest. The tea bags (d) were buried in the centre of the mesocosm (c). AM = Amiens (France), BR= Bremen (Germany), CH= Chimay (Belgium), EST= Southern Estonia, OR = Orléans (France), PR = Prignitz (Germany), SW = Southern Sweden, VL = Vlaanderen (Belgium).

**Table A3.3** the corresponding soil origin and treatment of plant communities.

| Region | Land use history | Soil type | Ctr | T | L | N | TL | TN | LN | TLN |
|---|---|---|---|---|---|---|---|---|---|---|
| AM | Ancient | Meso | Com12 | Com2 | Com9 | Com6 | Com10 | Com7 | Com1 | Com11 |
| | | Meso | Com8 | Com5 | Com11 | Com2 | Com9 | Com10 | Com3 | Com12 |
| | | Meso | Com3 | Com7 | Com2 | Com12 | Com1 | Com6 | Com9 | Com8 |
| | Post-agricultural | Eu | Com2 | Com7 | Com6 | Com1 | Com9 | Com12 | Com5 | Com3 |
| | | Eu | Com9 | Com6 | Com8 | Com2 | Com4 | Com3 | Com12 | Com5 |
| | | Meso | Com6 | Com5 | Com2 | Com7 | Com10 | Com9 | Com4 | Com8 |
| BR | Ancient | Oligo | Com10 | Com11 | Com9 | Com7 | Com12 | Com5 | Com6 | Com1 |
| | | Oligo | Com8 | Com10 | Com4 | Com3 | Com9 | Com12 | Com7 | Com2 |
| | | Oligo | Com10 | Com12 | Com7 | Com1 | Com11 | Com4 | Com6 | Com5 |
| | Post-agricultural | Oligo | Com4 | Com2 | Com5 | Com12 | Com1 | Com10 | Com9 | Com8 |
| | | Oligo | Com3 | Com1 | Com5 | Com6 | Com7 | Com8 | Com11 | Com9 |
| | | Oligo | Com3 | Com6 | Com10 | Com12 | Com7 | Com4 | Com8 | Com11 |
| CH | Ancient | Eu | Com6 | Com3 | Com11 | Com1 | Com10 | Com5 | Com7 | Com4 |
| | | Eu | Com5 | Com10 | Com6 | Com11 | Com2 | Com3 | Com1 | Com7 |
| | | Eu | Com11 | Com9 | Com1 | Com4 | Com12 | Com8 | Com3 | Com2 |
| | Post-agricultural | Eu | Com2 | Com4 | Com7 | Com3 | Com5 | Com12 | Com11 | Com1 |
| | | Eu | Com7 | Com4 | Com10 | Com6 | Com8 | Com2 | Com11 | Com5 |
| | | Eu | Com11 | Com9 | Com5 | Com7 | Com8 | Com10 | Com6 | Com4 |
| EST | Ancient | Meso | Com5 | Com2 | Com3 | Com9 | Com4 | Com6 | Com12 | Com1 |
| | | Oligo | Com1 | Com5 | Com6 | Com11 | Com3 | Com7 | Com4 | Com8 |
| | | Oligo | Com12 | Com6 | Com2 | Com11 | Com8 | Com5 | Com10 | Com7 |
| | Post-agricultural | Meso | Com9 | Com11 | Com1 | Com8 | Com5 | Com3 | Com12 | Com10 |
| | | Oligo | Com2 | Com6 | Com5 | Com4 | Com7 | Com11 | Com1 | Com3 |
| | | Oligo | Com5 | Com7 | Com6 | Com9 | Com11 | Com4 | Com8 | Com10 |
| OR | Ancient | Oligo | Com4 | Com7 | Com1 | Com3 | Com6 | Com8 | Com12 | Com11 |
| | | Oligo | Com12 | Com5 | Com8 | Com4 | Com2 | Com9 | Com11 | Com7 |
| | | Meso | Com6 | Com10 | Com12 | Com1 | Com3 | Com2 | Com4 | Com9 |
| | Post-agricultural | Oligo | Com9 | Com8 | Com3 | Com5 | Com7 | Com1 | Com4 | Com6 |
| | | Oligo | Com9 | Com12 | Com1 | Com8 | Com6 | Com11 | Com7 | Com2 |
| | | Meso | Com7 | Com11 | Com8 | Com4 | Com3 | Com2 | Com10 | Com9 |
| PR | Ancient | Oligo | Com10 | Com3 | Com11 | Com9 | Com12 | Com1 | Com8 | Com7 |
| | | Oligo | Com7 | Com8 | Com9 | Com12 | Com3 | Com4 | Com5 | Com6 |
| | | Oligo | Com12 | Com8 | Com7 | Com10 | Com6 | Com1 | Com9 | Com11 |
| | Post-agricultural | Oligo | Com11 | Com12 | Com2 | Com5 | Com1 | Com3 | Com9 | Com10 |
| | | Oligo | Com7 | Com9 | Com10 | Com11 | Com5 | Com6 | Com2 | Com3 |
| | | Oligo | Com4 | Com3 | Com10 | Com8 | Com12 | Com1 | Com2 | Com6 |
| SW | Ancient | Oligo | Com6 | Com11 | Com12 | Com9 | Com4 | Com8 | Com10 | Com1 |
| | | Meso | Com1 | Com4 | Com8 | Com2 | Com5 | Com9 | Com10 | Com3 |
| | | Oligo | Com2 | Com9 | Com3 | Com6 | Com11 | Com7 | Com5 | Com10 |
| | Post-agricultural | Oligo | Com5 | Com4 | Com12 | Com10 | Com1 | Com9 | Com3 | Com2 |
| | | Meso | Com1 | Com10 | Com9 | Com5 | Com4 | Com2 | Com8 | Com12 |
| | | Meso | Com4 | Com3 | Com12 | Com10 | Com8 | Com6 | Com7 | Com9 |
| VL | Ancient | Meso | Com3 | Com1 | Com11 | Com2 | Com10 | Com12 | Com5 | Com4 |
| | | Meso | Com1 | Com2 | Com7 | Com10 | Com11 | Com5 | Com3 | Com12 |
| | | Meso | Com8 | Com1 | Com3 | Com5 | Com6 | Com11 | Com2 | Com4 |
| | Post-agricultural | Meso | Com8 | Com12 | Com4 | Com3 | Com2 | Com10 | Com1 | Com5 |
| | | Meso | Com11 | Com1 | Com4 | Com8 | Com2 | Com7 | Com6 | Com12 |
| | | Meso | Com10 | Com8 | Com4 | Com7 | Com9 | Com11 | Com2 | Com6 |

AM = Amiens (France), BR= Bremen (Germany), CH= Chimay (Belgium), EST= Southern Estonia, OR = Orléans (France), PR = Prignitz (Germany), SW = Southern Sweden, VL = Vlaanderen (Belgium). A= Ancient forest, R = Recent forest (post-agricultural forest). Oligo = oligotrophic soil, Meso = mesotrophic soil, and Eu = eutrophic soil. Ctr = control (Ambient), T = Temperature, L = Light, N = Nitrogen.

**Figure A3.2** Scheme for the position of the plants (community 6) and teabags in the mesocosm. Four individuals per species were planted in each tray. Four teabags (two green tea and two rooibos tea) were buried at the intersection of four grid cells.

We measured the volumetric soil moisture content ($m^3.m^{-3}$) in all mesocosms after a dry period in September 2016 (13/09, no rainfall for 7 days) and after a rainfall event on October 3rd 2016. We used a Delta T ML3 Thetakit (Delta T, Cambridge UK), with two measurements in each mesocosm (one centre, one edge). We analysed the data using a linear mixed effects model with the volumetric soil moisture content as response, with rainfall event (2 levels), Soiltype (3 levels), Warming (2 levels), light addition (2 levels), N addition (2 levels), Land-use history (2 levels) as fixed main effects and the Plot number as the only random effect.

**Table A3.4** Mean volumetric soil moisture content ($m^3.m^{-3}$) in all mesocosms, analysed on main effects for rainfall event, soil type, land-use history. The intercept value is the soil moisture content on a dry day, on a “Eutrophic” soil with “Ancient” land-use history, and no warming, light or N addition applied.

| Term | Estimate | Standard error | T statistic | *p* value |
|---|---|---|---|---|
| (Intercept) | 41.17 | 0.87 | 47.36 | < **0.001** |
| Rainfall event | +1.64 | 0.33 | 4.99 | < **0.001** |
| Soil type | | | | |
| Mesotrophic | +1.81 | 0.54 | 3.32 | < **0.001** |
| Oligotrophic | -3.68 | 0.53 | -6.97 | < **0.001** |
| Land-use history | | | | |
| Post-agricultural | -3.33 | 0.36 | -9.26 | < **0.001** |
| Warming | -0.3 | 0.71 | -0.43 | 0.67 |
| Light | -0.79 | 0.71 | -1.10 | 0.27 |
| Nitrogen | -0.8 | 0.71 | -1.13 | 0.26 |

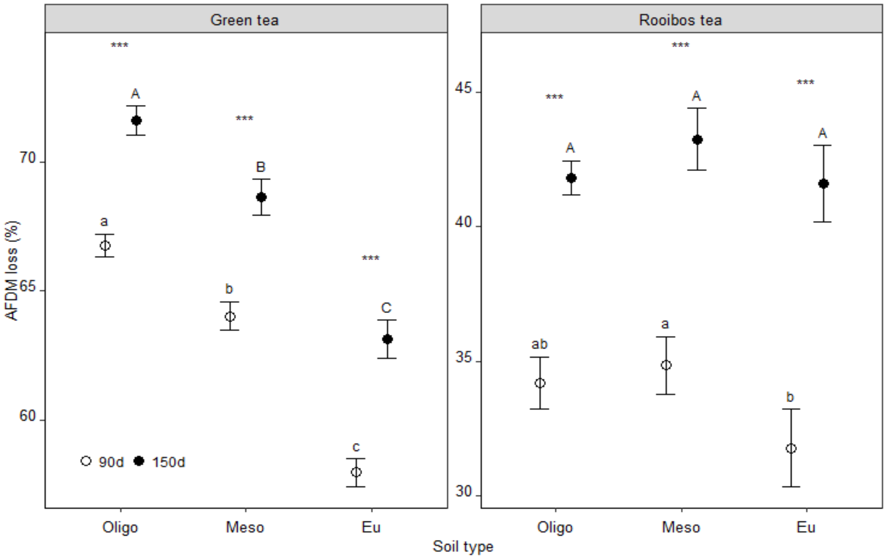

**Figure A3.3.** AFDM loss of green tea and rooibos tea after 90 and 150 days of incubation in oligotrophic soil (Oligo), mesotrophic soil (Meso) and eutrophic soil (Eu). Soils were collected in historically continuous 'ancient' forests and post-agricultural forests. Values are means with SE. Different letters indicate significant differences among soil type at the $p < 0.05$ level.

# CHAPTER 4

## Herbaceous litter determines macro-detritivores performance and tree litter decomposition

# Abstract③

The herbaceous layer comprises a large share of plant biodiversity in temperate forest. Yet, the functional role of herb litter on tree litter turnover and decomposers remains poorly understood. We conducted an experiment to determine the relative importance of macro-detritivores (woodlouse *Oniscus asellus* and millipede *Glomeris marginata*) and herb litter (*Aegopodium podagraria*) for the decomposition of five common tree species in temperate forest. In addition, due to the divergence in senescence time of summer herbs and tree leaves, the litter layer generally has a layered structure. Based on this, we also tested the role of the position of the herb litter on decomposition of tree litter, i.e. placing it above or below the tree litter. We found that the macro-detritivores increased tree litter mass loss, and macro-detritivore contribution increased with increasing tree litter quality. The herb litter also had significant effects on the mass loss of tree litter, but this effect depends on the position relative to the tree litter: positive effects on tree litter decomposition were found when herb litter was added above the tree litter, while the effect was often negative when it was added below the tree litter. However, in microcosms containing both detritivores and herb litter, the effect of herb litter on tree species was interfered by the presence of detritivores. This could be attributed to detritivore identity; *O. asellus* preferentially ingest herb litter, strongly reducing the quantity of herb litter (fragments) in the tree litter layer. The death rate of *G. marginata* was high in the presence of herb litter, possibly due to deterrent components in the herb litter or unfavourable microclimate that the herb litter created. Our study suggests that herb litter is important for tree litter turnover, especially if it covers the tree litter these effects are however mediated by detritivore identity.

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③ Wang, B., Verheyen, K., Baeten, L., & De Smedt, P. 2020. Herbaceous litter determines macro-detritivores performance and tree litter decomposition. *Pedobiologia*. (Under review)

## 4.1 Introduction

In terrestrial forest ecosystems, litter decomposition is the main driver in carbon and nutrient cycling, converting plant organic residues into small compounds or inorganic states (Austin & Vivanco 2006). Saprophagous macro-arthropods are known to play a vital role in litter fragmentation and biotransformation during the decomposition process, increasing the cycling of carbon and other nutrients (Hättenschwiler *et al.* 2005; David 2014). In general, soil detritivores can directly decompose litter through litter ingestion. Leaf parts are assimilated during the digestion processes and an unassimilated part is egested with their faeces, which is an important source of soil organic matter. On the other hand, the presence of detritivores may also affect the activity of other decomposers by increasing the physical disruption and dissemination of the microbial community on the litter surface, thus, indirectly affecting decomposition (Zimmer *et al.* 2005; David & Gillon 2009). In temperate forests, woodlice (Isopoda: Oniscidea) and millipedes (Diplopoda) are the most abundant above-ground macro-detritivores, responsible for 18% to 55% of the litter mass loss (Riutta *et al.* 2012). As plant litter is the main food source for these animals, the quality and quantity of it determines their performance (David & Handa 2010).

Woodlice and millipedes prefer litter with lower carbon: nitrogen (C:N) ratio, if not containing high levels of deterrent components (e.g. secondary compounds) (David & Handa 2010; Gerlach *et al.* 2014; De Smedt *et al.* 2018). As Gerlach *et al*. (2014) reported, when woodlice have a variety of food choices, they prefer to forage on high quality litter (with low C:N ratio), and avoid lower quality litter (e.g. *Fagus sylvatica*). Based on this information, low-quality monoculture tree litter may lead to malnutrition, reduced growth and reproduction, or mortality of detritivores due to starvation (Tranvik & Eijsackers 1989; Gannes *et al.* 1997), which may further slow down degradation rates. In this context, mixing litter with greater divergence of quality sturcture or wider litter heterogeneity provides a more diversified food choice compared to monocultures, or improves palatability of monoculture litter for decomposers because of the distinctly concentrations of nutrients among tree species (Pérez Harguindeguy *et al.* 2008; Gerlach *et al.* 2014; Fontana *et al.* 2019). Mixing different tree litter types has been shown to neutralize the physical and chemical properties of the recalcitrant litter in the mixture system (Gartner & Cardon 2004). However, tree leaves are not the only component on the forest floor litter layer; the herbaceous plants growing in the understorey also produce litter, which mixes with the tree litter on the forest floor.

Withered above-ground parts of herbaceous plants are generally characterized by a higher nutrient content (e.g. nitrogen, phosphorus, potassium, calcium and magnesium, Wang *et al.* 2020) and hydrophilic substance (e.g., protein and starch) (Fu *et al.* 2011), and are more palatable for most decomposers compared to tree litter (Chomel *et al.* 2016). This may have positive effects on the decomposition of tree litter when mixed. In accordance with tree-tree and herb-herb litter mixture studies, mixing herb and tree litter is expected to lead to physical, chemical, and biological changes in the decomposing system due to the great physicochemical heterogeneity (Pérez Harguindeguy *et al.* 2008). For instance, through the changes in water conservation, transfer of nutrients among litter types, and influences on the decomposer community (Tardif *et al.* 2014; Tardif & Shipley 2015; Grossman *et al.* 2020). One previous study in boreal forest plantations has indeed shown that mixing herbaceous litter with tree litter could enhance decomposition rates by maintaining higher humidity levels, by relieving N- or P-limitation, and by feeding a greater abundance of decomposers than single tree litter (Chomel *et al.* 2016).

Herbs in temperate forests are generally ephemeral species, mostly withering at the end of spring or summer, whereas the senescence of tree species is mostly in autumn (Jagodziński *et al.* 2016). This divergence in falling time leads to a vertical differentiation of litter on the forest floor, with the tree leaves generally falling on top of the herb litter. Given this vertical structure, there is probably only limited gravitational transfer of water and nutrients from herb to the tree litter. Moisture, nutrients, and microbes can be lifted through capillary rise from the soil towards the tree litter, the sandwiched herb litter could mediate this capillary rise. However, as a nutrient-rich material, the herb litter may attract decomposers to settle in herb litter layer but less spread to the above laying tree litter layer, thus, slowing down decomposition rates of tree litter. However, some recent studies have shown that the relative position of herb and tree litter could change in the context of global change (Vitasse *et al.* 2011; Marchin *et al.* 2015), resulting in unpredictable effects on tree litter decomposition. A change in the position of tree and herb litter (e.g. herb litter falls at the same time as the tree litter or even later) could have a stronger effect of herb litter on tree litter decomposition due to the transfer of herb fragments and nutrients via leaching (Gartner & Cardon 2004).

We performed a lab microcosm experiment to investigate the effects of herb litter addition, the relative position of the herb litter (above or below the tree litter), and the presence of two dominant macro-detritivores (woodlice and millipedes) on the decomposition of five common temperate deciduous broad-leaved tree species. Based on previous studies (see above), we expected that the presence of macro-detritivores increases tree litter decomposition and this effect increases with litter quality, as high-quality litter leads to higher animal performance. We hypothesised that: (i) adding herb litter increases tree litter decomposition and this effect is strongest with low quality tree litter and when macro-detritivores are present, as the herbaceous litter provides additional high quality food boosting the animals' performance; and (ii) the position of the herb litter mediates the effects of herb litter addition and macro-detritivore presence. We expect the strongest positive effect of herb litter addition when placed above the tree litter, due to gravitational transfer of herb litter fragments and nutrients towards the underlaying tree litter.

## 4.2 Material and methods

### 4.2.1 Litter Collection

We conducted a laboratory experiment to determine the effect of high-quality herbaceous litter, detritivores and their interaction on the decomposition of five common temperate tree species (*Quercus robur, Fagus sylvatica, Acer pseudoplatanus, Tilia platyphyllos*, and *Fraxinus excelsior*). Freshly fallen leaves were collected by litter traps, placed randomly in a mixed deciduous forest in northern Belgium (Aelmoeseneie forest, Gontrode, 50°58.5′ N, 3°48′ E, 16 m a.s.l.) in autumn 2017. The traps were emptied weekly until the end of the senescence period. *Aegopodium podagraria* was chosen as a dominant, summer withering herb species that generally shows a high nutrient concentration (Wang et al., 2020). Aboveground parts of *A. podagraria* were harvested in its senescence period (August 2018). All litter was air-dried for two weeks, then stored until the start of the experiment.

### 4.2.2 Experimental set-up

In September 2018, we filled 12 incubation trays (40*40*5 cm) with green floral foam (Oasis®). The floral foam, provides a moist environment to maintain litter moisture. We also constructed 106 microcosms using PVC pipe (Ø12 cm, depth 10 cm) covered with fiberglass gauze (1×2 mm mesh size; attached with cable ties to prevent detritivores from escaping) at the top and bottom. The fiberglass gauze allowed for moisture exchange, air circulation, and light penetration into the microcosms. Each microcosm was filed with 10 g of litter from one of the five tree species, which was oven-dried at 25°C for 24 h (to obtain even litter moisture but avoid litter structure damage by high temperatures).

The microcosms were assigned according to the treatments, litter of five tree species were used in this study (Fig. 4.1): control treatment (no herb litter and no detritivores), addition of detritivores, above and below addition of herb litter, addition of both herb litter and detritivores, herb litter only, and herb litter with detritivores. For the herb litter addition treatments, we added 6 g (equal to 5.5 g dried at 65 °C in oven) of *A. podagraria* litter. To investigate the effect of relative position of understorey herb litter on decomposition of tree litters, we applied the litter above (three of the five replicates per tree species) or below the tree litter (two of the five replicates per tree species). Tree litter and herb litter were separated by a circular net (Ø12 cm, 5×5 mm mesh size), allowing detritivores to pass. In order to investigate the feeding preference and effects of detritivores on *A. podagraria* litter and its effect on *A. podagraria* decomposition. For the detritivore addition treatments, we added four woodlice (*Oniscus asellus*, a drought sensitive species) and four millipedes (*Glomeris marginata*, a drought tolerant species) (De Smedt *et al.* 2018). The detritivores were hand collected in the same forest as where the litter was collected. In order to prevent a sudden increase of juveniles during the experiment, we excluded pregnant females (with brood pouch). The individuals were weighted separately, and then were sorted into three categories by weight (large, medium and small). The detritivores were kept for maximum two weeks in a plastic box filled with a substrate composed of leaf litter and some soil from where animals were collected before adding them to the microcosms. To reduce the divergence in initial weight of detritivores in each microcosm, we added one large, two medium, and one small individual to each mesocosm (Fig. A4.1).

The leaves in the microcosms were sprayed with a microbial wash before detritivore addition, to restart microbial activity on the leaf litter after drying. The microbial wash was created by soaking the ectorganic horizon from the litter collecting site for a few days. Particles were then filtered out (0.02 mm mesh size of the filter) and the solution was sprayed on the microcosms one day before detritivores were added. This should mimic natural colonization of microbes immediately at the start of the experiment. The incubation trays were also filled with this microbial wash until the floral foam was saturated. Finally, the microcosms were randomly distributed over the incubation trays (3 by 3 in each tray), and transferred into two climate chambers. A daily cycle of 12 h of daylight and 12 h of darkness, with a constant temperature of 20°C was maintained in the climate chamber for the whole incubation period. During the experiment, floral foam moisture and litter moisture was kept constant gravimetrically with tap water, the water was sprayed on top of the microcosms every 3-5 days, the last watering was 4 days before the sampling. The experiment started on October 15, 2018 and ended January 23, 2019 a period of around three months (100 days).

### 4.2.3 Measurements

After 50 days of incubation in the climate chambers, we checked the survival of the detritivores. We retrieved the microcosms after 100 days of incubation, the detritivores were sorted out and were weighed again individually. Tree litter and herb litter samples were separately weighted before and after oven-dried (at 25°C for 24 h) to determine the moisture content and mass loss during the incubation.

### 4.2.4 Statistical analyses

The litter moisture percentage was calculated as [(Fresh weight – Dried weight) / Dried weight] * 100. We tested the effect of the herb litter positions on moisture of both herb and tree litters by applying one-way analysis of variance (ANOVA) on tree and herb moisture data, and a Tukey's HSD post *hoc* test for pairwise differences. The effect of tree species, the herb litter addition and detritivores addition on mass loss of tree litters were tested with three-way ANOVA. Tukey's HSD post *hoc* test was preformed to explore differences between tree species and the position treatments of herb litter. We performed these tests separately for the subset of mesocosms where the herb litter was added above versus below the tree litter. The effect of the herb litter treatments (no addition, below the tree litter, and above the tree litter) on detritivore survival after 50 and 100 days of incubation was tested with ANOVA and Tukey's HSD post *hoc* tests for pairwise comparisons. The survival between the two incubation times (50 and 100 days) within a treatment were also tested using t-test. Finally, the effect of the herb litter treatments and tree species on detritivore weight changes before and after the experiment was tested with ANOVA and Tukey's HSD post *hoc* tests. All these statistical analyses were performed using the software package R version 3.0.3 (R Core Team, 2018).

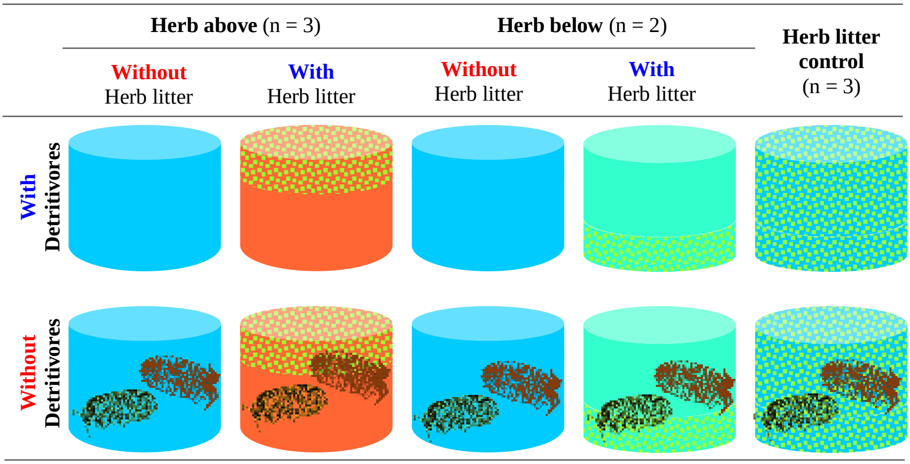


**Figure 4.1** Schematic overview of the experimental set-up.

## 4.3 Results

### 4.3.1 Litter moisture content

The moisture level of tree litter was significantly higher in the treatment with herb litter than the control ($p < 0.05$), especially when the herb litter was added below the tree litter (Fig. 4.2, Fig. A4.2).

### 4.3.2 Tree litter mass loss

After 100 days of incubation, we observed that the labile litters (e.g. *A. pseudoplatanus*, *T. platyphyllos* and *F. excelsior*) had about 2-times higher mass loss than the more recalcitrant litters (*F. sylvatica* and *Q. robur*). Adding detritivore species without herb litter (i.e. controls in Fig. 4.3) generally increased litter mass loss. The herb addition effect was, however, dependent on the position of the litter and the presence of detritivores. In the absence of detritivores, all tree litters showed a significant higher mass loss when the herb litter was added above the tree leaves (Fig. 4.3, Table 4.1). On the contrary, adding herb litter below the tree litter decreased or did not show an effect on the tree litter mass loss. When detritivores were added, however, the positive effect of adding herb litter above was not as consistent and only clearly positive for *Acer pseudoplatanus* and *Fraxinus excelsior* (Fig. 4.3).

**Table 4.1** Summary table for three-way ANOVAs testing the effects of the detritivore (D) and the herb litter (H) addition treatments and the differences between the tree species (TS) on the tree litter mass loss. The test was done separately for the subset of mesocosms where the herb litter was added above versus below the tree litter. Significant *p* values are highlighted in bold.

| Factor | *Df* | Herb litter above the tree litter | | | | Herb litter below the tree litter | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Sum-Sq | Mean-Sq | *F* | *p* | Sum-Sq | Mean-Sq | *F* | *p* |
| Detritivores (D) | 1 | 0.121 | 0.121 | 16.944 | **< 0.001** | 0.318 | 0.318 | 42.000 | **< 0.001** |
| Herb litter (H) | 1 | 0.529 | 0.529 | 74.107 | **< 0.001** | 0.786 | 0.786 | 103.709 | **< 0.001** |
| Tree species (TS) | 4 | 7.118 | 1.779 | 249.459 | **< 0.001** | 5.439 | 1.360 | 179.508 | **< 0.001** |
| TS: D | 4 | 0.254 | 0.064 | 8.899 | **< 0.001** | 0.170 | 0.043 | 5.623 | **< 0.001** |
| TS: H | 4 | 0.126 | 0.032 | 4.433 | **0.002** | 0.318 | 0.080 | 10.490 | **< 0.001** |
| D: H | 1 | 0.217 | 0.217 | 30.462 | **< 0.001** | 0.001 | 0.002 | 0.194 | 0.660 |
| TS: D : H | 4 | 0.151 | 0.038 | 5.282 | **< 0.001** | 0.096 | 0.024 | 3.170 | **0.014** |
| Residuals | | 2.283 | 0.007 | | | 2.045 | 0.008 | | |

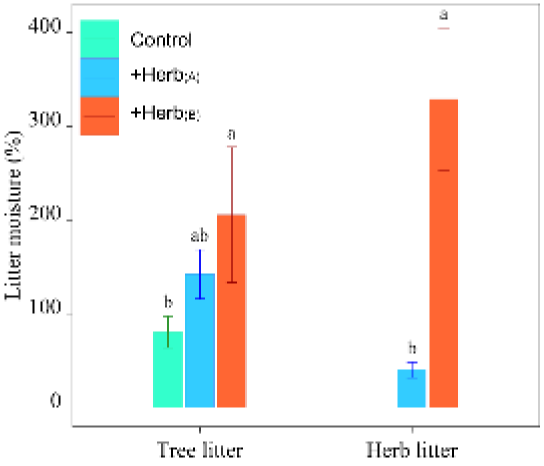


**Figure 4.2** Tree and herb litter moisture percentage depending on the herb litter positions (above (+Herb$_{(A)}$) or below (+Herb$_{(B)}$) the tree litter). For the tree litter, the control for tree litter represent mesocosms without herb litter addition, the control for herb litter represent the mesocosms with herb litter only. Each value is the mean ± 1 standard error.

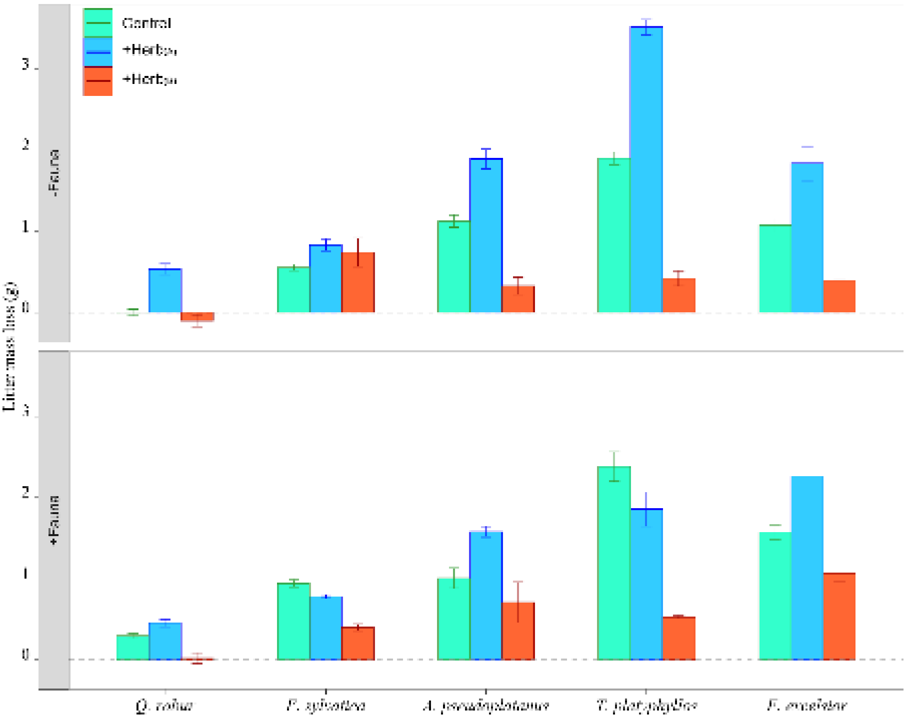


**Figure 4.3** Mass loss of tree litter in relation to a detritivore treatment (no detritivores “-Fauna” and detritivores added “+Fauna”) and a herb litter addition treatment (no herb litter addition “control”, herb litter added above of “+Herb$_{(A)}$”, and below “+Herb$_{(B)}$” the tree litter). Tree species are sorted from recalcitrant litter (left) to labile litter (right). Each value is the mean ± 1 standard error. Statistical differences between the treatments and tree species are provided in Table 1.

When the herb litter was added underneath the tree litter, it still decreased the tree litter decomposition. Interestingly, we observed some inhibitory effects of herb addition on the liable tree litters. The presence of detritivores increased the consumption of the herb litter below the recalcitrant litters by 48% to 88% (Fig. A4.3). In the absence of detritivores, mass loss of the herb litter below the liable tree litters was significantly higher than the herb litter below recalcitrant tree litters (Fig. A4.3).

### 4.3.3 Detritivores survival and weight change

Mortality was high during the early stages (first 50 days) of incubation for woodlice (56 ± 8% survival) and this mortality was consistent across the treatments. Millipedes had much higher survival rate (95± 2%) in the first 50 days in the tree litter control treatment, but adding the herb litter significantly reduced survival to 53.3% (addition on top of tree litter) and 32.5% (addition below tree litter, Fig. 4.4). After 100 days of incubation, additional mortality of both woodlice and millipedes was observed, but the herb litter treatment effects remained the same. The woodlice in the microcosm with *A. pseudoplatanus* and herb litter had mostly died (Fig. A4.4).

**Table 4.2** Summary table for two-way ANOVAs for the effects the herb litter (H) addition treatment and tree species (TS) on weight changes of (a) woodlice and (b) millipedes. The test was done separately for the subset of mesocosms where the herb litter was added above versus below the tree litter. Significant *p* values (< 0.05) are highlighted in bold.

| | Herb above | | | | | Herb below | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Df* | Sum-Sq | Mean-Sq | *F*-value | *p* | *Df* | Sum-Sq | Mean-Sq | *F*-value | *p* |
| **(a) Woodlice** | | | | | | | | | | |
| Herb litter (H) | 4 | 0.000 | 0.000 | 0.316 | 0.867 | 3 | 0.000 | 0.000 | 0.329 | 0.804 |
| Tree species (TS) | 1 | 0.003 | 0.003 | 7.454 | **0.007** | 1 | 0.001 | 0.001 | 1.786 | 0.185 |
| H: TS | 3 | 0.001 | 0.000 | 1.119 | 0.345 | 3 | 0.001 | 0.000 | 0.610 | 0.611 |
| Residuals | 108 | 0.037 | 0.000 | | | 90 | 0.036 | 0.000 | | |
| **(b) Millipedes** | | | | | | | | | | |
| Herb litter (H) | 4 | 0.008 | 0.002 | 2.380 | 0.054 | 4 | 0.010 | 0.003 | 2.877 | **0.025** |
| Tree species (TS) | 1 | 0.001 | 0.001 | 0.762 | 0.384 | 1 | 0.003 | 0.003 | 3.068 | 0.082 |
| H: TS | 4 | 0.017 | 0.004 | 4.971 | **0.001** | 4 | 0.004 | 0.001 | 1.135 | 0.343 |
| Residuals | 154 | 0.129 | 0.001 | | | 129 | 0.117 | 0.001 | | |

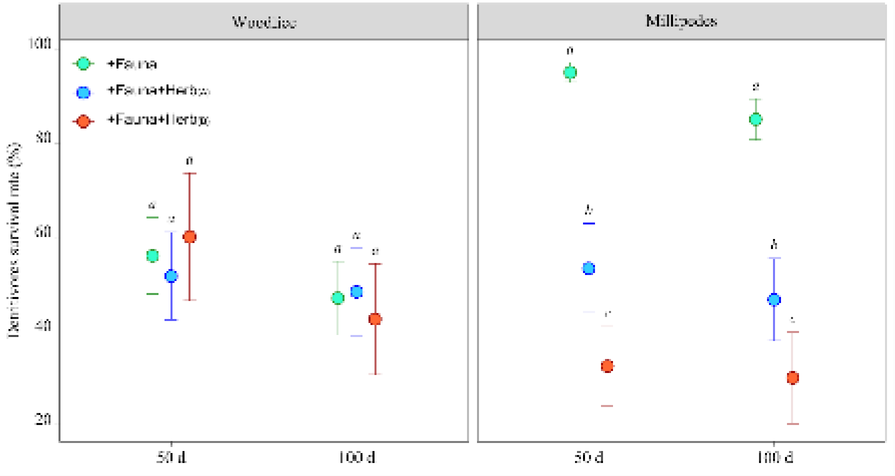


**Figure 4.4** The survival rate of woodlice (a) and millipede (b) after 50 and 100 days of incubation in litter with and without herb litter addition. Different letters (a, b, c) show significant differences between the herb litter treatments within an incubation period and taxonomic group. Points represent mean survival ± 1 standard error.

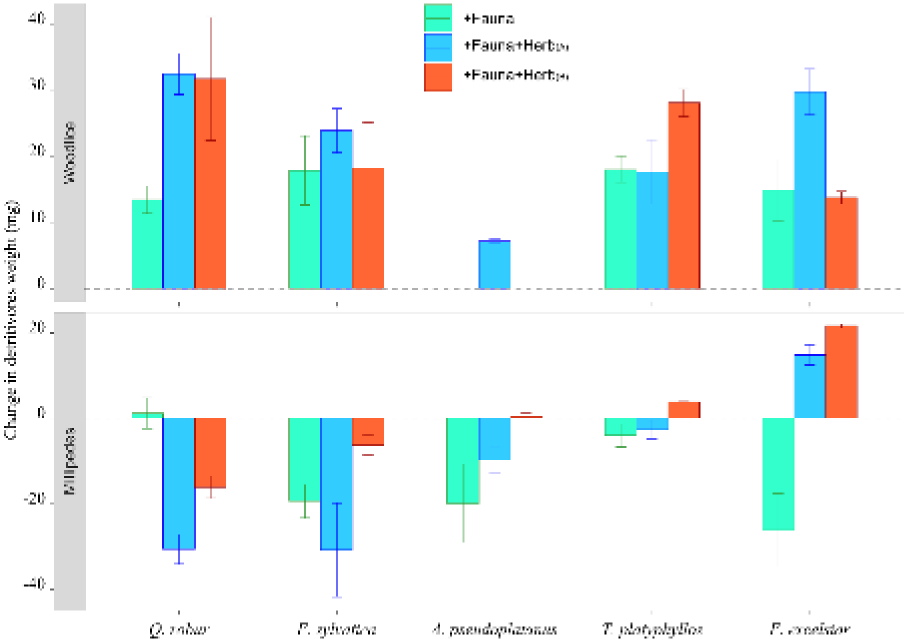


**Figure 4.5** The mass change of woodlice and millipedes in different types of tree litter and herb litter treatments: no herb litter addition "+Fauna", herb litter added above of "Fauna+Herb$_{(A)}$", and below "Fauna+Herb$_{(B)}$" the tree litter. Tree species are sorted from recalcitrant litter (left) to labile litter (right). Each value is the mean ± 1 standard error. Statistical differences between the herb litter treatments and tree species are provided in Table 2.

After 100 days of incubation, the weight changes of woodlice and millipedes showed opposite patterns (Fig. 4.5). On average, woodlice weight increased with 8.09 ± 0.49 mg, while the weight of millipedes decreased with 0.59 ± 0.40 mg across detritivore size classes (there was no difference in weight gain and survival between the size classes, Fig. A4.4, A4.6) and litter types. Adding herb litter above as well as below both had significant effects on the weight of detritivores (Table 4.2). Compared to the control, herb litter addition led to 3.95 ± 3.64 and 17.43 ± 5.76 mg of weight increase of woodlice when the herb was added above and below tree litter, respectively. The millipedes under control lost 13.67 ± 7.76 mg of weight on average. The effect of herb litter on millipede weight changes were related to tree litter quality. In liable litters, weight changes were not as negative (or even positive in *F. excelsior*) compared to the control. This mitigation effect was not found in recalcitrant litter types (*Q. robur* and *F. sylvatica*).

## 4.4 Discussion

High quality litter is expected to accelerate litter decomposition rates of recalcitrant litter, due to the maintenance of higher litter moisture and nutrient transfer from high to low quality litter (Madritch & Cardinale 2007; Jonsson & Wardle 2008). Additionally, adding higher quality litter to recalcitrant litter might also support the performance of arthropod detritivore species, which are critical in the decomposition process, possibly increasing the litter consumption (Coulis *et al.* 2013; Fontana *et al.* 2019). In this study, we investigated the effects of herb litter addition and arthropod detritivore presence on the decomposition of the leaf litter of five common temperate European tree species in an experimental mesocosm study. The mass loss of tree litters (except the *A. pseudoplatanus* litter) significantly increased when macro-detritivores were present in the microcosm, confirming our first hypothesis, where we hypothesized that the presence of macro-detritivores increases tree litter decomposition. In line with our second hypothesis, adding herb litter increased tree litter decomposition, however, this was not consistently positive but clearly depended on its relative position to the tree litter (above or below) and the presence of detritivores, in spite of our expectation that the herbaceous litter provides additional humidity and high-quality food boosting the animals' performance. Finally, as expected in our third hypothesis, the position of the herb litter clearly influenced the decomposition of the tree litter, with generally positive effects when added above the tree litter.

### 4.4.1 The effect of detritivores on decomposition

Macro-detritivores play a crucial role in the decomposition of dead plant material in terrestrial ecosystems. The annual consumption rate of woodlice and millipedes is about 14 g litter per individual per year (David & Gillon 2002). During our 100-day laboratory experiment, the mass loss of all tree litter species under the control treatment was lower compared to the tree litter under the detritivore treatment. This result was similar to the results (at 50 mg level of body size) reported by David & Gillon (2002). The absence of a clear positive effect of detritivore addition on maple (*A. pseudoplatanus*) decomposition was probably related to the surprisingly high mortality of woodlice (100% death at the beginning of incubation) in these mesocosms. We could not observe a high consumption rate of woodlice on *Acer* litter as found by Hättenschwiler and Bretscher (2001) and De Smedt *et al.* (2018). The reason for the mass mortality of woodlice in the microcosm with maple litter might be due to the physical characteristics of the leaves. The leaves are very broad and almost completely filled the microcosms. A possible cause of mortality could be that woodlice (represented by a drought sensitive species that prefers to retreat to the bottom of the microcosm, but has a relatively low inundation resistance when submerged (Ooms *et al.* 2020), could not escape from underneath the leaves and drowned when we watered the litter.

Based on the weight changes, it is likely that the positive effects of detritivore addition on the decomposition of both liable and recalcitrant litters is mostly due to the woodlice species and not to the millipede species. This is in accordance with a previous study reporting that woodlice play a comparatively more important role than millipedes in the decomposition processes for certain litter types (Vos *et al.* 2011). The weight changes of millipedes showed a reverse trend to that of woodlice and these opposite results are not likely due to the food competition between two detritivores species, since they performed well in similar experiments with even higher densities (De Smedt *et al.* 2018). In addition, the millipedes still lost weight in the microcosm with *A. pseudoplatanus* litter in which almost all woodlice died. The mass loss of *A. pseudoplatanus* litter did not differ between the with- and without-detritivores treatment, partly revealing the higher importance of woodlice compared to millipedes for litter decomposition. The litter consumption rates could be even higher if there would not have been a mass death of woodlice (56 ± 8%) at the start of the experiment, which was also found by Vos *et al.* (2013). This might probably be due to low moisture levels when installing the microcosms.

The effects of detritivores on litter decomposition clearly depends on the tree litter quality (De Smedt *et al.* 2018). Woodlice and millipedes typically show a higher propensity to high quality food source, which are generally rich in nitrogen (David & Handa 2010). In our research, the *T. platyphyllos* and *F. excelsior* litter has higher nitrogen concentrations than *F. sylvatica, Q. robur,* and *A. pseudoplatanus* (Wang *et al.* 2020), so the former species were more palatable for the macro-detritivores. The detritivores consumed nearly twice as much of liable litter types compared to the recalcitrant litter types, while, neither woodlice nor millipedes gained more weight when they were living in liable litter compared to recalcitrant litter.

Previous studies have found that detritivore effects could be amplified in mixed litter, when the mixture litter provides a higher diversity or higher quality of nutrients, making monoculture litter more palatable for the decomposers (David & Handa 2010). At the same time, detritivores could give preference to high quality litter when both nutrient-rich and -poor litters are present in the same ecosystem (Vos *et al.* 2011). Our study supports such selection effects, as we found that there was in general no positive effect of detritivores on the breakdown of tree litter when adding herb litter. A potential explanation is that due to the feeding preferences of the detritivores, the moist and nutritious environment in the herb layer might be favoured by decomposers (both microbial and saprophagous fauna) reducing tree litter consumption rates (Gerlach *et al.* 2014).

### 4.4.2 The effect of herb litter on decomposition

Presence of herb litter clearly regulated the diet of detritivores, but the role of herb litter on decomposition of tree litters goes further than this. Our results showed that the herb litter also increased moisture content of tree litter. Changes in litter moisture retention capacity is a reason for the acceleration of decomposition rates in mixed litter, leading to a series of direct and indirect effects on litter decomposition processes (Hättenschwiler *et al.* 2005; Hoorens *et al.* 2010). In line with these findings, we found that the tree litter with herb litter addition maintained higher moisture content than without herb litter addition, in particular when herb litter was added below the tree litter (Fig. 4.1). Benefited from the hydrophilic matters leaching and evaporation blocking of the above herb litter, the three litters below the herb litter had slightly higher moisture (Deutsch *et al.* 2010; Wang *et al.* 2016).

However, in contrast with our hypothesis and some other studies (Riutta *et al.* 2012; Makkonen *et al.* 2013; Petraglia *et al.* 2019), the decomposition of tree litter with the two-fold moisture content (when the herb litter was sandwiched between soil and tree litter layer) was not faster compared to the tree litter with medium moisture (due to the above addition of herb litter). In fact, a positive effect of herb litter on litter decomposition was only found when the herb was added above tree litters, while negative when the herb litter was sandwiched between soil and tree litter layer. One could argue that the moisture availability was probably not the limiting factor for litter decomposition in our experiment and the effects of herb litter position are driven by other factors. The on-top addition of herb litter to tree litter possibly influenced the decomposition of tree litter via nutrient transfer (Wang *et al.* 2020; Chomel *et al.* 2016). Here, when the herb litter was added above the tree litter, the hydrophilic substances and nutrients transfer happened through leaching, thus, the moisture and nutritional conditions in the tree litter layer becomes more favourable for decomposers, and further, stimulating microscopic decomposition. In addition, enhanced microbial activity could also enhance feeding quality for macro-detritivores (Vos *et al.* 2011).

While the herb litter species we used is likely to delight microbial decomposers, and some macro-arthropods, the millipede species in our experiment clearly suffered from the addition of *A. podagraria* litter. Based on the survival of millipedes, it looks like the summer herb litter is detrimental to a certain extend for this millipede species. There are two potential explanations for the mass mortality of millipedes when the summer herb litter was added (either added above or below the tree litter) in the microcosm. High mortality might be due to high amounts of deterrent components (e.g. toxins, tannins, phenolics or silica) in the summer herb (Zimmer 1997; Gramss & Bergmann 2008; Foss *et al.* 2013; Hartley & DeGabriel 2016). These prevent millipedes from feeding on the herb litter and subsequently via leaching also on the tree litter until some of these components are leached out and are not detrimental anymore to *G. marginata*. Secondly, the environmental conditions in the mesocosms where the herb litter was added could force millipedes to live in unsuitable conditions, leading to sickness and higher mortality. For example, the absence of sun light in the climate chamber (the light in climate chamber was supporting by cool-white fluorescent bulbs, but not natural sunlight) and the increased moisture when adding the herb litter might be too wet for the studied millipedes (as a drought tolerant species) (De Smedt *et al.* 2018). The humid environment could also lead to the breeding of harmful bacteria for the millipedes, often resulting in sickness and mortality (Bastow *et al.* 2008).

The relative position of herb litter and tree litter provided opposing results. We found a synergistic mixture effect when the herb litter was placed above the tree litter, whereas it showed an antagonistic effect when the herb litter was placed below the tree litter, despite the below addition of herb litter improving the moisture content of the tree litter. This result implies that the relative senescence period of tree and herb species might change the soil C and nutrient stocks. The aboveground part of *A. podagraria* generally wither earlier than most of deciduous tree species in temperate forests (Jagodziński *et al.* 2016), which means the *A. podagraria* litterfall in natural forests generally leads to an antagonistic effect on tree litter, as it is mostly positioned below the tree litter. However, the synergistic effects of overlaying *A. podagraria* litter on decomposition of tree litter could be observed in *Betula pendula* or *Populus trichocarpa* forests, because these two species generally wither earlier than most other deciduous tree species in temperate forests. On the other hand, the relative position of *A. podagraria* litter and tree litter could be placed in the context of global change (Vitasse *et al.* 2011; Marchin *et al.* 2015). The turnover rates of organic matter on forest floor would be further accelerated (already being accelerated by the warming) by overlaying herb litters (Gartner & Cardon 2004).

In summary, our work verified the importance of marco-detritivores and herbaceous litter for the decomposition of five common temperate broad-leaved deciduous species. Our results clearly showed that the decomposition of tree litter is positively affected by the presence of macro-detritivores, woodlice in particular, and the contribution of macro-detritivores on breakdown of liable litters was higher than on recalcitrant litters. The contribution of herb litter to tree litter decomposition clearly depended on both tree litter quality and the vertical position of the herb and tree litter. The decomposition of tree litter was stimulated when herb litter was added above tree litter. On the contrary, it was inhibited when the herb litter was sandwiched between tree litter and soil layer, even though the herb litter led to a higher moisture content of the tree litter. The presence of herb litter influenced macro-detritivores' feeding preference for tree litter, based on litter type and litter position and detritivore identity. Overall, our results illustrate that understorey herbs in interaction with litter feeding macro-detritivores can have an important role in determining organic matter turnover and nutrient release in temperate forest.

## Acknowledgments

BW was supported by the China Scholarship Council while studying at Ghent University (No. 201606910080). PDS holds a post-doctoral fellowship of the research foundation Flanders (FWO).

# Appendix

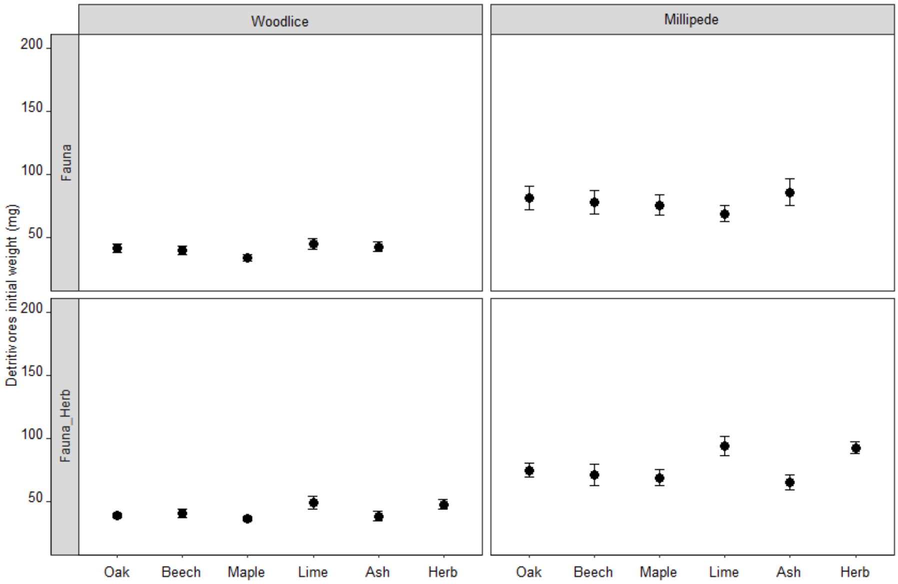


**Figure A4.1** Average initial weight of woodlice and millipedes in different litters and treatments.

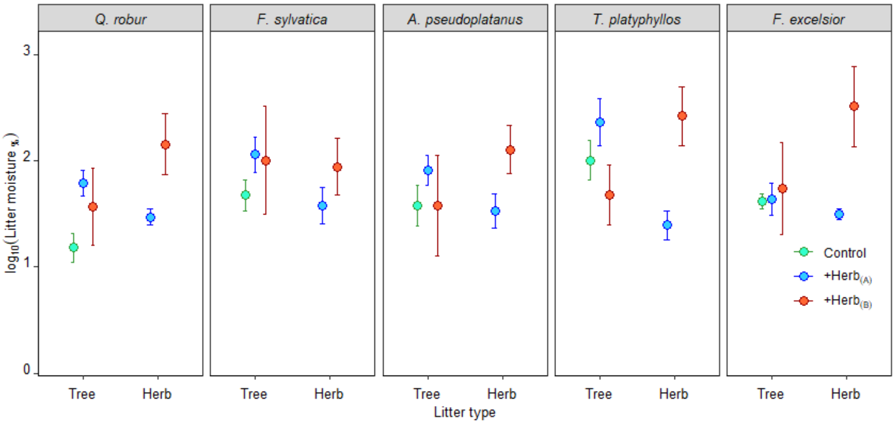


**Figure A4.2** Moisture content (lg10 data) of tree and herb litters in different microcosms with six type of litters.

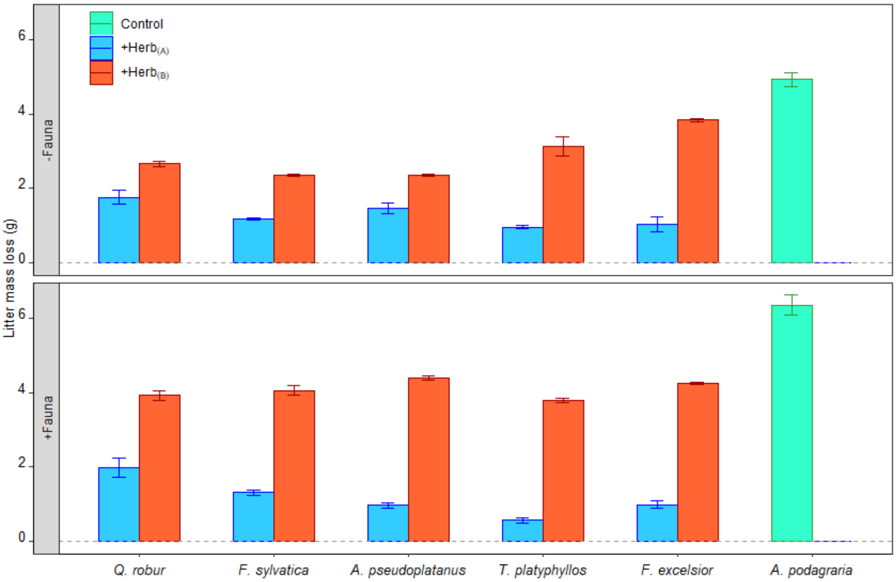


**Figure A4.3** Mass loss of herb litter responds to the position treatments of herb litter. "Herb$_{(A)}$" and "Herb$_{(B)}$" means the position of the herb litter is above and below the tree litters, respectively. Each value is the mean ± 1 standard error.

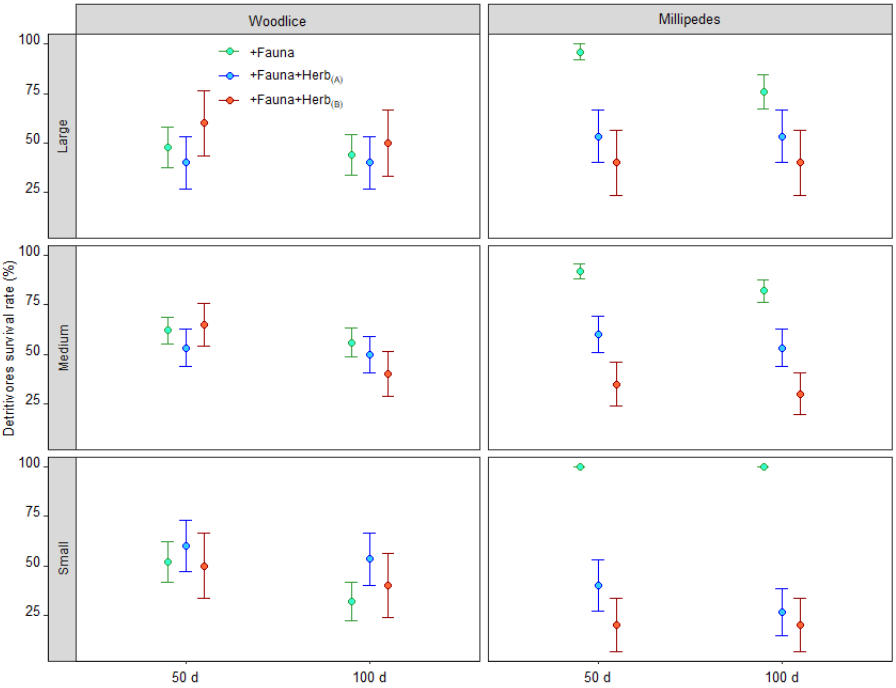


**Figure A4.45** Survival of three types of woodlice and millipedes at 50- and 100-days of incubation. Each value is the mean ± 1 standard error.

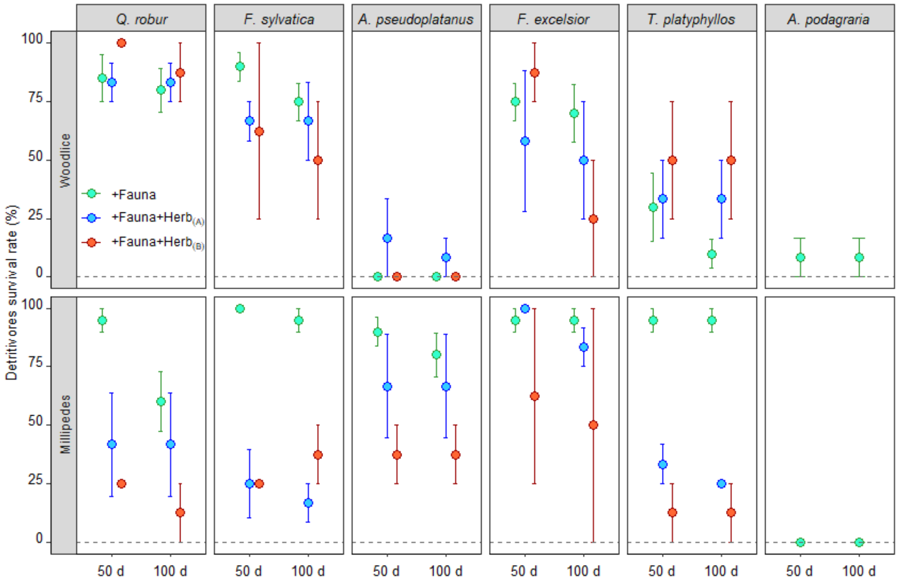


**Figure A4.5** Survival of woodlice and millipedes in six type of litters. Each value is the mean ± 1 standard error.

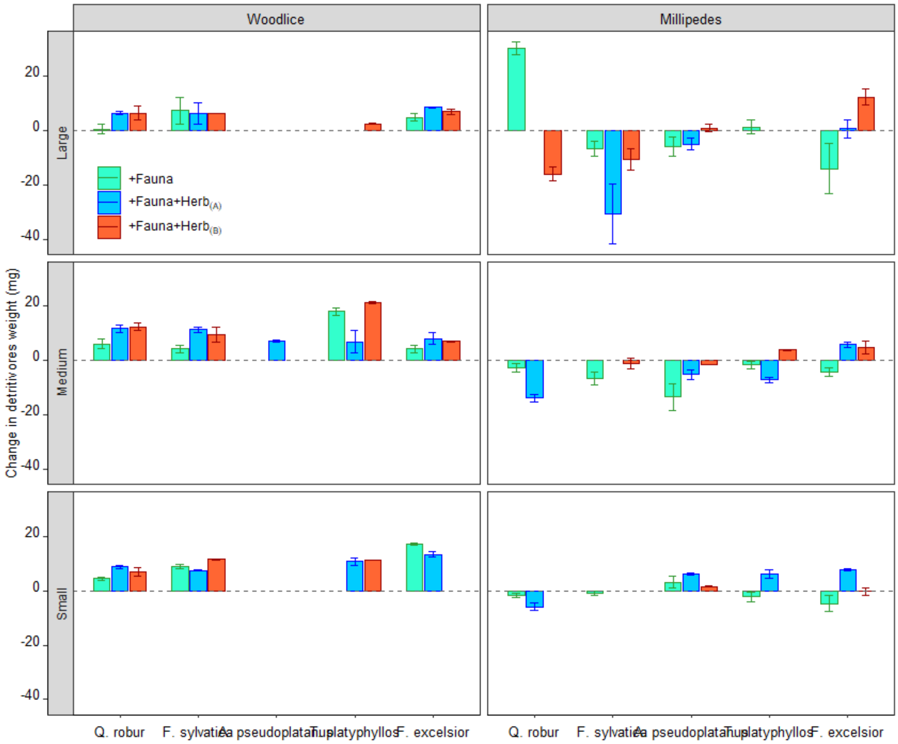


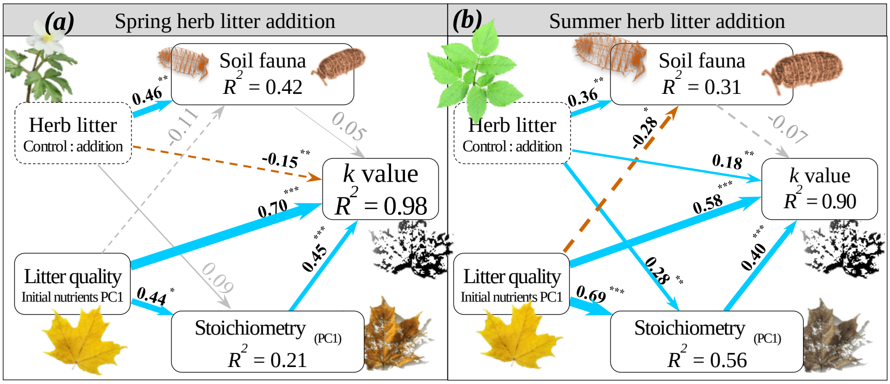
**Figure A4.6**. The mass changes of three types of woodlice and millipedes in different types of tree litters with or without herb litter addition. Each value is the mean ± 1 standard error.

# CHAPTER 5

# Herb litter mediates tree litter decomposition and soil fauna composition

# Abstract④

Leaf litter from forest understorey species has very different characteristics (e.g. a higher nutrient content) which are regarded to be more palatable and digestible for the decomposer community compared to litter from the overstorey trees. Consequently, the decomposition of tree litter may show different dynamics when mixed with litter from herb species. To study the effect of understorey litter on tree litter decomposition, we set up a litterbag experiment in a temperate mixed deciduous forest in Belgium. We investigated the effect of spring (*Anemona nemorosa*) and summer (*Aegopodium podagaria*) herb litter on the litter decomposition of five native tree species that differ in litter quality. The spring and summer withering herb litters were added above the tree litters. After 742 days of in situ incubation, mass loss (40% to 100%) and the decomposition rate (0.27 to 1.68 yr-1) of the litter from the five tree species were positively correlated with litter quality. The addition of summer herb litter resulted in increased mass loss and decomposition rates (*k*) of the underlying recalcitrant tree litter. In addition, summer herb litter increased nutrient concentration in the short-term as well as the abundance of soil fauna during litter decomposition. In contrast, spring herb litter slightly inhibited tree litter decomposition. Structural equation models showed that the effect of understorey litter on decomposition rates of tree litter acted via nutrient leaching towards the tree litter layer rather than via the alteration of soil fauna (detritivores and omnivores) abundance. Our results show that litter from understorey vegetation can alter nutrient availability in recalcitrant litters (*Fagus sylvatica* and *Quercus robur*) via increased nitrogen (N), calcium (Ca), magnesium (Mg), phosphorus (P) and potassium (K) concentrations by 9%, 21%, 22%, 41%, and 315% respectively after the summer herb litter addition. The effect on tree litter decomposition strongly depends on herb species identity and future research should focus on the generality of the observed patterns. We conclude that herb litter has a major role in accelerating carbon sequestration and nutrient cycling in temperate forests.



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④ Adapted from Wang, B., Verheyen, K., Baeten, L., & De Smedt, P. 2021. Herb litter mediates tree litter decomposition and soil fauna composition. *Soil Biology and Biochemistry*, 152, 108063.

## 5.1 Introduction

Litter decomposition plays an essential role in the functioning of terrestrial ecosystems (Austin & Vivanco 2006; García-Palacios *et al.* 2016b). Important drivers of litter decomposition are the decomposer community (i.e., micro-organisms and soil fauna), the abiotic environment (e.g., temperature, soil moisture, and soil pH), and litter quality (i.e., physical structure and chemical composition) (Coûteaux *et al.* 1995; Cornwell *et al.* 2008). These three factors can explain up to 70% of the variation in decomposition rates in terrestrial ecosystems (Trofymow *et al.* 2002; Parton *et al.* 2007). Previous research on litter decomposition in temperate forests has focussed mainly on the dynamics of litter from a single tree species. Although increasing attention has been paid to the influence of mixing the litter of tree species on decomposition (Berger & Berger 2014; Setiawan *et al.* 2016), the role of understorey herb species litter on the decomposition rate of tree litter has largely been ignored.

The influence of mixed litter on decomposition can be driven by individual plant characteristics or by a combination of physical, chemical, and biological processes in the litter layer (Gartner & Cardon 2004; Wu *et al.* 2014; Setiawan *et al.* 2016). Mixing leaf litter from species that differ in litter quality and structure changes the physiochemical properties of the decomposition environment, for instance via nutrient transfer from high-quality (labile) litter to low-quality (recalcitrant) litter (Madritch and Cardinale 2007). Bonanomi et al. (2014) reported, for instance, a highly positive mixture effect for litter breakdown when mixing two types of litter that differed strongly in their N-content, indicating that mixing high-quality and low-quality litter together can increase the decomposition rate of the low-quality litter. Mixing high quality litter, which typically has a high content of hydrophilic substances, with low quality litter (e.g., high C:N ratio and a low content of macronutrients) could also improve the humidity levels of the decomposition environment compared to when the low quality litter decomposes alone (Deutsch *et al.* 2010).

Additionally, synergistic effects of mixtures could be indirectly caused via soil meso- and macro-fauna communities. Soil fauna are known to play a key role in litter decomposition (Hansen & Coleman 1998; Wu *et al.* 2014; Setiawan *et al.* 2016). As an important functional group, detritivores (and omnivores) perform a crucial first step in the process of breaking down litter through ingestion and assimilation of leaf litter. Any unassimilated litter is egested with faeces, which is an important source of soil organic matter. Predatory (or omnivorous) soil fauna can regulate the structure and activity of decomposer communities (e.g., detritivores and the microbial community) on the litter surface, thus, indirectly affecting decomposition (Zimmer *et al.* 2005; David & Gillon 2009). Litter mixtures, especially mixtures in which the litter quality varies widely, are expected to have a higher abundance and richness of decomposers (soil microbes and soil fauna) compared with litter from monocultures because they create a more suitable habitat and a complementary food source for the decomposers (Gartner & Cardon 2004). For example, Wu et al. (2014) and Wardle et al. (2006) recorded a higher microbial biomass and soil fauna diversity in litter mixtures compared with monocultures.

The herbaceous understorey community has long been considered an unimportant 'step-over' layer in forests because of its relatively low biomass and limited competition for nutrients compared to the overstorey vegetation of shrubs and trees (Gosz *et al.* 1972; Gilliam 2007). However, a recent study has shown that the understorey has important effects on the functioning of forest ecosystems (Landuyt *et al.* 2019). Among other effects, the understorey vegetation can alter soil physicochemical properties (i.e., soil porosity, pH, and nitrification), due to its root system and through litter production (Matsushima & Chang 2007; Xiong *et al.* 2008; Fu *et al.* 2015). More specifically, herbaceous forest species are generally characterized by high nutrient concentrations, which are on average between 1.5 and 5 times (up to even 30 times for potassium) greater than overstorey tree leaves (Welch *et al.* 2007; Zhao *et al.* 2017). According to existing theories on mixture effects, mixing herbaceous litter with tree litter could therefore have strong stimulatory effects on tree litter decomposition (Chomel *et al.* 2016).

In temperate forests, the period of time in which litter from herbaceous understorey plants falls varies considerably between species. Litter fall depend on the species' phenology and range from early spring to late summer or autumn. The annual litter input of the herbaceous understorey can be up to 20% of annual litter input in forest ecosystems and this litter will become available for decomposition throughout the growing season, which is in contrast to the dominant litterfall period of overstorey species in the autumn (Eisenhauer *et al.* 2010; Landuyt *et al.* 2019). Consequently, a large proportion of herbaceous litter will fall on top of the previous year's autumn tree litter, possibly altering the decomposition of this tree litter (Chomel *et al.* 2016). Freshly fallen litter from understorey herbaceous species may enrich the physicochemical properties of the tree litter by leaching herb litter fragments and nutrients into the underlying tree litter. This enrichment of tree litter can also attract additional decomposers since most of its nutrients (important food source for decomposers) may already be utilized during the early stages of tree litter decomposition (Berg 2014). Litter from herbaceous species can therefore attract more soil fauna (both in quantity and diversity and of different trophic levels, e.g., predators, detritivores, and omnivores) to the litter layer. The increased presence and activity of soil fauna may increase litter consumption and damage the surface structure, further enhancing the decomposition process of the tree litter by meso and micro fauna and micro-organisms (Hättenschwiler & Gasser 2005; A'Bear *et al.* 2013; Smith *et al.* 2019).

To investigate the effects of the addition of herb litter on tree litter decomposition, we performed a litterbag experiment in a mixed temperate deciduous forest. We tested the effect of two dominant herbaceous understorey species with contrasting phenologies (spring vs. summer ephemerals) to explore the possible influence of the timing of the high-quality litter inputs. Five common European tree species (common beech (*F. sylvatica* L.), pedunculate oak (*Q. robur* L.), sycamore maple (*Acer pseudoplatanus* L.), ash (*Fraxinus excelsior* L.), and large-leaved lime (*Tilia platyphyllos* Scop.)) were used to represent a gradient in chemical litter quality. We hypothesize the following: (i) that decomposition of tree litter is enhanced by adding high-quality herbaceous litter and that this effect would be the greatest for recalcitrant (low-quality) tree litter, compared with labile tree litters, due to the greater contrast in litter quality (Bonanomi et al. 2014, Liu et al. 2020); (ii) nutrient concentrations of the tree litter change by adding herb litter, due to the nutrient transfer from the added herbaceous litter (Madritch & Cardinale 2007); and (iii) the addition of herb litter will increase the abundance and diversity of soil fauna, such as detritivores and omnivores, which contributes to the enhanced decomposition of (especially) the recalcitrant litters (Hättenschwiler & Gasser 2005).

## 5.2 Material and methods

### 5.2.1 Site description

The study was performed in an old-growth temperate mixed deciduous forest in Gontrode, Belgium (East-Flanders, 50°58.5′ N, 3°48′ E, 16 m a.s.l.). The forest has a total area of 39.5 ha and the oldest trees are about 100 years old. The annual precipitation is *ca.* 850 mm and is evenly distributed throughout the year. The mean annual temperature is 11.3°C, with around -0.5°C in the coldest months (January and February) and around 18.5°C in the warmest months (July and August). Beech (*F. sylvatica* L.) and pedunculate oak (*Q. robur* L.) dominate and are interspersed with less abundant tree species such as sycamore maple (*A. pseudoplatanus* L.), ash (*F. sexcelsior* L.) and large-leaved lime (*T. platyphyllos* Scop.). European rowan (*Sorbus aucuparia* L.), European hazelnut (*Corylus avellana* L.) and alder buckthorn (*Frangula alnus* Mill.) are commonly found in the shrub layer. The most abundant herbaceous species includes wood anemone (*Anemone nemorosa* L.), Ground elder (*Aegopodium podagraria* L.) and Lesser celandine (*Ficaria verna* Huds.) (Blondeel *et al.* 2019). Soils in this forest are Retisols and

Stagnosols (World Reference Base (WRB) for soil resources), which have a typical thin quaternary layer of sandy loam with a spotted texture B-horizon on a shallow impermeable clay and sand complex of tertiary origin. The humus layer is of mull and moder type.

### 5.2.2 Litter collection

We collected freshly fallen leaves from the study site during the litter fall period in 2016 (October to November). We used 50 circular nylon net litter traps, which were 1 m high and 0.55 m in diameter. The litter traps were randomly placed under the forest canopy to avoid contamination of the litter with soil. The traps were emptied weekly until the end of the litter fall period. We collected ca. 30 kg of fresh fallen (mixed) leaves. The tree litter was homogenized and dried at room temperature for two weeks. Then the litter was dried at 25 °C in an oven for 24 h to obtain homogeneous litter quality but avoid damage to the litter structure from high temperatures. We separated the tree litter by species: *F. sylvatica, Q. robur, A. pseudoplatanus, T. platyphyllos,* and *F. excelsior*. Litter from all other species were removed. *A. nemorosa* and *A. podagraria,* which naturally falls at the end of spring and summer, respectively, were chosen as herbaceous litter because of the strong difference in senescence period between the two species. The aboveground parts of *A. nemorosa* and *A. podagraria* were harvested at the end of their growing season right before they fell on the forest floor in June and October, 2017, respectively, and the leaves were dried following the same methods for tree litter described above.

### 5.2.3 Experimental set-up

The litterbags were constructed from 20 × 20 cm nylon nets with a mesh size of 5 × 5 mm to allow microorganisms and soil fauna to access the bags and contribute to the decomposition process (Dilly *et al.* 2001; Yin *et al.* 2019). Each litterbag was filled with the litter of one species: one of the five tree species or one of the two herb species.

For tree litter, we added oven-dried (25 °C, not totally dried) tree litter in a bag, corresponding to 10 g oven-dried mass (65 °C) according to the litter moisture. A total of 435 tree litterbags were prepared and divided into the following four groups (Fig. 5.1):

- **A:** control group with 165 bags that were placed on the forest floor (on 21st December, 2016) and to be sampled on eleven dates, spanning from the start to the end of the experiment (five tree species × eleven samplings × three replicates);

- **B:** spring herb addition group; a total of 120 litterbags were placed on the forest floor (on the same date as group A) for (above) adding the spring herb litter (on 2nd June, 2017), which were sampled eight times after the spring herb addition (five tree species × eight samplings × three replicates with spring herb litter addition);
- **C:** summer herb addition group; 75 litterbags were placed on the forest floor (on the same date as group A) for adding summer herb litter (on 10th October, 2017), which were sampled five times after the summer addition (five tree species × five samplings × three replicates);
- **D:** spring plus summer herb addition group; 75 litterbags were placed on the forest floor (on the same date as group A) for adding both spring (the same date as group B) and summer (the same date as group C) herb litters, which were sampled five times after the summer addition (five tree species × five samplings × three replicates).

Each group represented a time series beginning at different time points, depending on the herb litter type that was added. For instance, in group B, the 120 litterbags containing litter from different tree species were placed in the field at the same time as groups A, C, and D (on 21st December 2016), but litterbag sampling only started 2 weeks (15th June, 2017) after spring herb litter was added (2nd June, 2017), hence resulting in (three) fewer sampling dates (and litterbags). The decomposition of the tree litter in this group before the first sampling date (and thus before any addition of herb litter) can be inferred from the bags collected in control group A, which were put in the field at the same time and also did not receive any additional litter. All litterbags were fixed with four nails on the surface of the soil, with one replicate in each of the three randomly selected plots (4 × 7 m in size and at least 10 m away from each other). The herb layer and freshly fallen litter were removed from the ground prior to placing the litterbags.

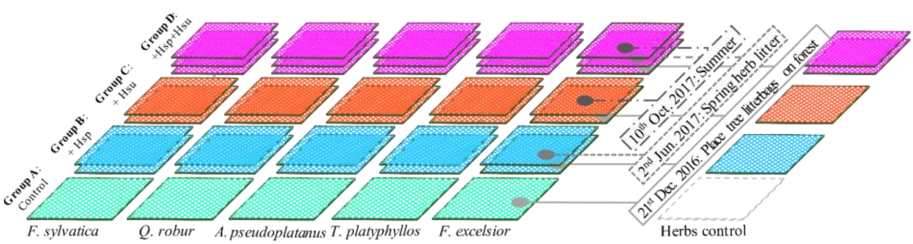

**Figure 5.1**. Schematic overview of the set-up of the litterbag series. The litter bags with tree litter were all placed on the forest floor on 21st December, 2016, the litter bags with spring and summer herb litter were added above the tree litters on 2nd June and 10th October, 2017, respectively. Control = tree litter only, +Hsp = spring herb litter addition, +Hsu = summer herb litter addition, +Hsp+Hsu = spring and summer herb litter addition.

Litterbags with herbaceous litter were filled with 10 g oven-dried (25 °C) herb litter in similar nylon bags as used for the tree litters. On 2nd June 2017, the bags filled with spring herb litter were placed on top of the bags containing tree litter that were placed in the field in the previous winter (21st December, 2016) in group B and group D. A total of 234 litterbags containing litter from the spring herb species were used [144 bags of spring herb litter for group B: (five bags were placed above five tree species + one bag for spring herb control) × eight sampling dates × three replicates; and 90 litterbags for group D: (five bags were placed above five tree species + one for spring plus summer herb control) × five sampling dates × three replicates]. On 10th October 2017, the bags with summer herb litter were fixed on top of the tree litter in group C and group D. A total of 180 summer herb litterbags were used in the summer herb litter addition treatments [90 summer herb litterbags for group C: (five bags were placed above five tree species + one for summer herb control) × five sampling dates × three replicates; and 90 for group D: (five bags were placed above five tree species + one for spring plus summer herb), respectively].

Immediately after all of the litterbags were placed in the forest, three litterbags of each tree species were randomly collected (day 0) for initial chemical tests. Three randomly selected litter bags were also collected when the herb litter was placed in the forest. On each sampling date (between January 2017 to January 2019), one litterbag per species was collected randomly from each of the three replication plots. Litter bags in group A (control group) were collected after 22, 98, 163, 176, 203, 293, 307, 334, 372, 483, and 741 days of incubation (11 collections). For group B (spring herb addition), collection started 2 weeks after the addition of spring litter, on day 176 (based on the timeline of group A), and then followed the same sequence as group A (a total of eight collections were made). Similarly, after 293 days of incubation, summer herb litter was added to group C and D. Samples were collected from groups C and D on day 307, after the addition of the summer herb litter. The litterbags containing tree litter, spring herb litter, and summer herb litter were collected. The final collections were done after 307, 334, 372, 483, and 741 days after placement in the field. The collected litterbags were stored in plastic bags and transferred to the laboratory for immediate processing.

### 5.2.4 Fauna extraction and chemical analysis of litter

To extract soil fauna, the collected litterbags were first opened and added to Tullgren-funnels (with 5×5 mm mesh size) to extract soil fauna for three days (Carrillo *et al.* 2011). Soil fauna samples were stored in 70% alcohol and identified to order or subclass level.

For four fauna orders we separated larvae from adults since they represent very distinct functional groups and are easily recognised (i.e. Coleoptera, Diptera, Lepidoptera and Neuroptera). The fauna collections were categorised into five groups (detritivores, omnivores, predators, phytophages, and xylophages) according to their feeding strategy. Afterwards, leaf litter was oven-dried at 65 °C for 2 days to a constant weight. Following drying, roots, seeds, flowers, and shells not belonging to the species included in the litter were removed by hand, and the adhered soil particles were removed through using a 1 mm mesh sieve. The remaining mass was weighed.

Litterbags with tree and herb litter for initial chemical traits (day 0), the first collection after the addition of spring litter (day 176), the first collection after the addition of summer herb litter (day 308), and the second to last collection (day 483) were mixed at the replication level. Afterwards, the samples were ground (used mill: Retsch ZM1, Germany) and used for chemical analysis. A CNS elemental analyser (vario Macro Cube, Elementar, Germany) was used to measure C and N. The concentration of P, K, Ca, and Mg were measured with flame atomic absorption spectrophotometry (Varian SpectrAA-240) after digestion with 2 mL $HNO_3$ and 0.4 mL of $HClO_4$ to a 75 mg subsample in Teflon pots for 4 h at 140 °C. Samples were then diluted up to 50 mL (Blondeel *et al.* 2018). The lignin and cellulose content are important determinants of litter quality.

**Table 5.1** Initial chemical composition and C:N ratio in freshly fallen tree litters in autumn and two types of herbaceous litters (n = 1).

| Litter type | Species | C (g $g^{-1}$) | N (mg $g^{-1}$) | P (mg $g^{-1}$) | K (mg $g^{-1}$) | Ca (mg $g^{-1}$) | Mg (mg $g^{-1}$) | C:N |
|---|---|---|---|---|---|---|---|---|
| Tree litter | *F. sylvatica* | 0.51 | 13.14 | 0.56 | 5.36 | 8.51 | 1.26 | 38.92 |
| | *Q. robur* | 0.50 | 15.07 | 0.70 | 4.66 | 9.11 | 1.38 | 32.87 |
| | *A. pseudoplatanus* | 0.47 | 16.61 | 0.56 | 6.81 | 14.23 | 2.01 | 28.10 |
| | *T. platyphyllos* | 0.47 | 19.78 | 0.94 | 4.52 | 18.31 | 2.71 | 23.78 |
| | *F. excelsior* | 0.47 | 22.34 | 1.86 | 9.71 | 26.46 | 4.30 | 21.22 |
| Herb litter | *A. nemerosa* | 0.44 | 27.36 | 1.94 | 18.98 | 18.91 | 4.41 | 15.93 |
| | *A. podagraria* | 0.41 | 21.52 | 4.98 | 42.37 | 14.08 | 3.40 | 19.22 |

The chemical composition of the litter showed that the litter from the tree and herb species represent a clear quality gradient (Table 5.1, Fig. A5.1). The litter of *F. sylvatica* and *Q. robur* had a higher initial C content and C:N ratio than the other species; whereas the nutrient content was 1.5 to 9 times higher in *T. platyphyllos*, *F. excelsior*, and the herb litter. Litter from *A. pseudoplatanus* had the highest P (49.76 mg $g^{-1}$) and K (42.37 mg $g^{-1}$) concentrations of the litter collected from the five tree species. A principal component analysis (PCA) on the initial chemical composition (C, N, P, K, Ca, and Mg) of the five tree species and two herbaceous species used in this study (Fig. A5.1) showed that first principal component (representing 72% of total variance) can be considered the major axis of variation in litter chemistry, varying from low to high-quality litter. This axis is used in the data analysis.

### 5.2.5 Data analysis

The effect of tree species (five levels), herb litter addition treatment (four levels), and their interaction on the remaining mass of tree litter was tested with two-way ANOVA. Tukey's HSD post hoc tests were performed to explore differences in the remaining mass between the litter species and herb litter addition treatments. Due to the strong non-linearity of the changes in litter mass remaining over time, we did not analyse the data as a time series with collection date as continuous predictor. Instead, analyses were performed separately for the different collection dates (i.e., 308, 336, 385, 483, and 742 days of incubation) after the addition of litter from the summer herbaceous species.

The decomposition rate ($k$) was calculated by fitting the observed data to the exponential decay model as described by Olson (1963):

$$M_t = M_0\, e^{-kt} \qquad (1)$$

where $M_t$ and $M_0$ are the litter mass remaining at collection data $t$ and the initial mass, respectively, and $k$ is the decomposition rate (expressed as $yr^{-1}$). Decomposition rates were calculated for each tree species and herb litter addition treatment combination (three replicates at each of the collection dates). Next, we used a two-way analysis of variance (ANOVA) to evaluate the effects of tree species, the addition of herb litter type, and their interaction on the decomposition rate.

The effects of tree species, herb litter addition treatment, and their interaction on carbon and nutrient release was tested with a two-way ANOVA and Tukey's HSD post-hoc test. Nutrient stocks (mg) were calculated by multiplying nutrient concentrations with the remaining dry mass at 176, 307, and 483 days after incubation. The same effects were tested on the soil fauna communities in tree litter. Response variables were the total number on individuals and the diversity of functional groups present (Shannon-Wiener index) (Shannon & Weaver 1963).

$$H = -\sum_{i}^{S} p_i \ln(p_i) \qquad (2)$$

where *pi* is the proportional of the ith taxa (*Ni*) to the total number of all individuals (*N*) in the sample, *S* is the total number of soil fauna groups.

Graphs were produced using *ggplot2* (Bodenhofer *et al.* 2011). All analyses were done in R package version 3.0.3 (R Core Team 2018).

In addition, nonmetric multidimensional scaling (NMDS; Euclidean dissimilarity) was used to visualize the variation in the soil fauna community among the different treatments, providing an ordination of fauna associated with the litterbag experiments over a two-dimensional space. To test for compositional differences of the fauna communities between the tree species and herb litter treatments (multivariate location effects), we applied a permutational multivariate analysis of variance using the *adonis* function in the R vegan package (version 2.5-6). Differences in the variation within the treatment combinations were determined with analysis of multivariate homogeneity of group dispersions using the *betadisper* function with 999 permutation in the R *vegan* package (Oksanen *et al.* 2019).

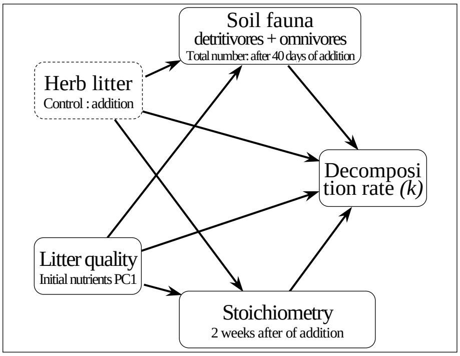

**Figure 5.2** A *priori* conceptual structural equation model depicting pathways by which tree litter quality, herb litter treatments, abundance of soil fauna decomposers, and remaining stoichiometry in decomposing tree litters may influence litter decomposition rates (*k*).

To synthesise effects, we constructed two separate structural equation models (SEM) that examined the direct effects of spring or summer herb litter on the soil fauna community, nutrient composition, and decomposition of tree litter, and the indirect effects of herb litter, via their impacts on the biotic (soil fauna community) and abiotic (nutrient transfer) processes during tree litter decomposition (Fig. 5.2). Before building the models, we reduced the dimensionality of the litter nutrient concentration data using a PCA to derive a principal axis of variation in litter quality (see 'chemical analysis of litter' and Fig. A5.1 in supporting information) (García-Palacios *et al.* 2016a). Considering the functional role of soil fauna, we only used the total number of detritivores and omnivores in our SEM. We used the *piecewiseSEM* package in R to implement the models, and a Fisher's *C* test to retain the hypothesised relationship structure (Lefcheck 2016). The data was considered to fit the path model when the *P*-value for Fisher's *C* statistic was > 0.05 (Shipley 2009). Due to the interaction of spring and summer herb litter on tree litter decomposition, the data from the treatment of "spring+summer herb litter addition" was removed in the SEM models.

## 5.3 Results

### 5.3.1 Mass loss and decomposition rates of litter

The herb litter treatment significantly affected the remaining mass for each of the collection dates following the addition of summer herb litter ($p < 0.001$, Table 5.2, Fig. 5.3). The effect of the addition of herb litter also tended to increase over time. Looking at patterns within the tree species, the decomposition of the recalcitrant litter types (*F. sylvatica*, *Q. robur*, *A. pseudoplatanus*) slowed when adding spring (+ summer) litter compared with the control and especially compared with the summer herb litter addition treatment (Fig. 5.3). There was no significant effect of spring or summer herb litter addition on the decomposition of *T. platyphyllos* and *F. excelsior*, as both species decomposed almost completely in all herb litter experiments. The decomposition rate *k* according to Olson's single exponential model was significantly influenced by the addition of herb litter ($F = 4.83$, $p = 0.006$, Table A5.1). The decomposition rates of tree litter (especially for *F. sylvatica*, *Q. robur,* and *A. pseudoplatanus* litter) in the spring and spring+summer herb litter treatments were lower compared to the control (Table 5.3). In contrast, the addition of summer herb litter generally increased tree litter decomposition rates (Table 5.3). Specifically, the decomposition rates of *F. sylvatica, Q.* r*obur*, and *F. excelsior* litter under the summer herb addition treatment were increased by approximately 74%, 90%, and 67%, respectively, but the decomposition rates did not differ for *Q. robur*.

**Table 5.2** Effect of tree species (TS), litter treatment (T) and their interaction (TS:T) on litter remaining mass at five different days of incubation, corresponding to the litter bag collection dates after the summer herb litter was added. Effects were tested with ANOVA. Significant *P* values are highlighted in bold.

| Factor | *df* | 308 days | | 336 days | | 385 days | | 483 days | | 742 days | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *F* | *p*-value | *F* | *p*-value | *F* | *p*-value | *F* | *p*-value | *F* | *p*-value |
| Tree species (TS) | 4 | 193.29 | **<0.001** | 183.83 | **<0.001** | 164.63 | **<0.001** | 179.88 | **<0.001** | 157.15 | **<0.001** |
| Treatment (T) | 3 | 4.435 | **0.009** | 3.888 | **0.016** | 7.576 | **<0.001** | 10.566 | **<0.001** | 10.639 | **<0.001** |
| TS:T | 12 | 0.679 | 0.761 | 0.990 | 0.475 | 2.087 | **0.041** | 1.368 | 0.221 | 2.088 | **0.041** |

**Table 5.3** Effect of tree species and herb litter treatment on decomposition rate (*k*) of tree litter from five tree species (values are mean ± se). Different letters show significant differences within treatments (lower-case letters) and within species (capital letters). Effects were tested with ANOVA with Tukey's HSD post-hoc tests. Control = tree litter only, +Hsp = spring herb litter addition, +Hsu = summer herb litter addition, +Hsp+Hsu = both spring and summer herb litter addition.

| Treatment | *F. sylvatica* | *Q. robur* | *A. pseudoplatanus* | *T. platyphyllos* | *F. excelsior* |
|---|---|---|---|---|---|
| Control | 0.27 ± 0.04 $^{\text{c B}}$ | 0.38 ± 0.02 $^{\text{c A}}$ | 1.08 ± 0.06 $^{\text{b AB}}$ | 1.58 ± 0.10 $^{\text{a A}}$ | 1.69 ± 0.15 $^{\text{a C}}$ |
| +Hsp | 0.19 ± 0.03 $^{\text{c B}}$ | 0.26 ± 0.04 $^{\text{c A}}$ | 0.65 ± 0.06 $^{\text{bc B}}$ | 1.57 ± 0.41 $^{\text{ab A}}$ | 2.40 ± 0.24 $^{\text{a BC}}$ |
| +Hsu | 0.48 ± 0.08 $^{\text{c A}}$ | 0.73 ± 0.04 $^{\text{bc A}}$ | 1.34 ± 0.19 $^{\text{bc A}}$ | 1.54 ± 0.34 $^{\text{b A}}$ | 2.78 ± 0.16 $^{\text{a AB}}$ |
| +Hsp+Hsu | 0.21 ± 0.02 $^{\text{c B}}$ | 0.27 ± 0.20 $^{\text{c A}}$ | 0.64 ± 0.06 $^{\text{bc B}}$ | 1.62 ± 0.06 $^{\text{b A}}$ | 3.06 ± 0.15 $^{\text{a A}}$ |

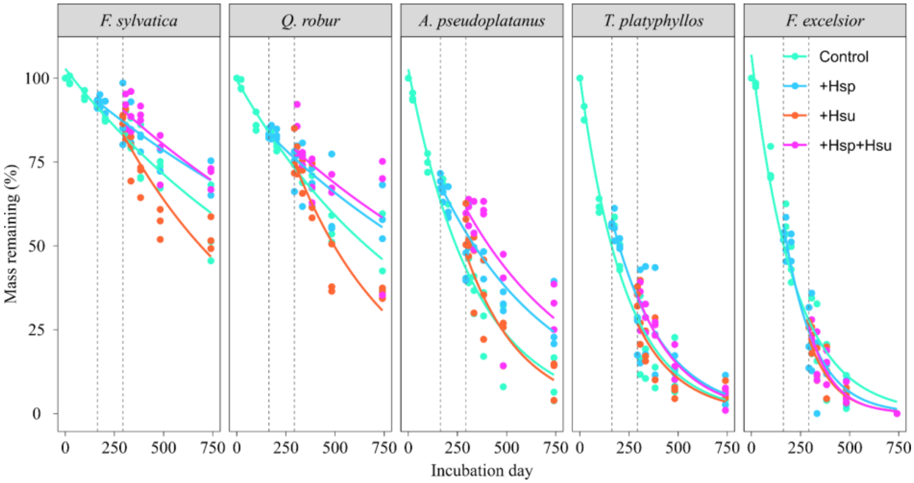

**Figure 5.3**. Remaining litter mass of the studied tree species under different treatments of herb litter addition at eleven collection dates. The lines show the exponential decay model for each litter addition treatment, based on the mean decomposition rate (*k*) of three replicates. Vertical lines indicate when the spring and/or summer herb litter was added. Control = tree litter only, +Hsp = spring herb litter addition, +Hsu = summer herb litter addition, +Hsp+Hsu = both spring and summer herb litter addition.

### 5.3.2 Carbon and nutrient release

Over 481 days of incubation, the total stocks of C, N, P, K, Ca, and Mg of the litter from all tree species decreased over time (Fig. A5.2); however, the pattern was different if we consider the concentration of the elements. The concentration of K decreased over time for all tree species, whereas the concentration of N, P, and Mg generally showed an increasing trend. The C concentration of the leaf litter of most species, except *F. excelsior*, decreased over time. The concentration of Ca in recalcitrant litter showed a generally increasing trend with the increasing number of incubation days, but a decreasing trend was observed in *F. excelsior* (Fig. 5.4). The addition of summer herb litter generally led to a temporary increase in the K concentrations in the litter from the five tree species, which increased three to five times in tree litter 14 days after the addition of summer herb litter. Patterns for other nutrients were generally weaker and more variable. Across the litter from the tree species, the addition of summer herb litter had positive effects on the concentration of K, P, Mg, and N of tree litter (Fig. 5.4) and the concentrations increased by 424%, 21%, 16%, and 3%, respectively. On the other hand, the addition of herb litter (especially summer herb litter) generally led to lower C concentrations in tree litter (except *Q. robur* litter) compared with the control. Moreover, the herb litter treatments had a stronger effect on the C and nutrient concentration in the labile litter (e.g., *T. platyphyllos* and *F. excelsior* litter) than in the recalcitrant litter (e.g., *F. sylvatica* and *Q. robur*).

### 5.3.3 Soil fauna

During the 742 days incubation period, a total of 40,385 individuals were extracted from the five types of tree litter, 4,935 from the spring herbaceous litter, and 10,878 from the summer herbaceous litter. The fauna community included 19 taxonomic groups, which we classified into five functional groups (Table A5.2). The most predominant taxa in quantity were Acari and Collembola, accounting for 52% and 28% of the total number of individuals, respectively. The addition of herb litter had significant effects on the total number of soil fauna in tree litter ($F = 17.403$, $p < 0.001$, Table A5.3 and A5.4). After the addition of spring litter, the total number of soil fauna (between days 176–293) increased, especially in the labile litter, e.g., *T. platyphyllos* ($F = 4.477$, $p = 0.050$). In contrast, after the addition of summer litter, the total number of soil fauna increased significantly in recalcitrant litter, e.g., *Q. robur* ($F = 3.03$, $p = 0.044$, Fig. 5.5). The diversity (Shannon-Wiener index) of soil fauna in *Q. robur* litter underneath the herb litter increase significantly ($F = 3.463$, $p = 0.028$). The multivariate NMDS analyses revealed differences between detritivore and omnivore communities associated with the herb litter treatments and tree litter types ($p_{adonis} = 0.007$, $p_{dispersion} = 0.014$, $p_{adonis} = 0.009$, Fig. A5.4a, b). The herb litter treatments differed significantly in the compositional variation of the fauna communities within the treatments (Fig. A5.4a).

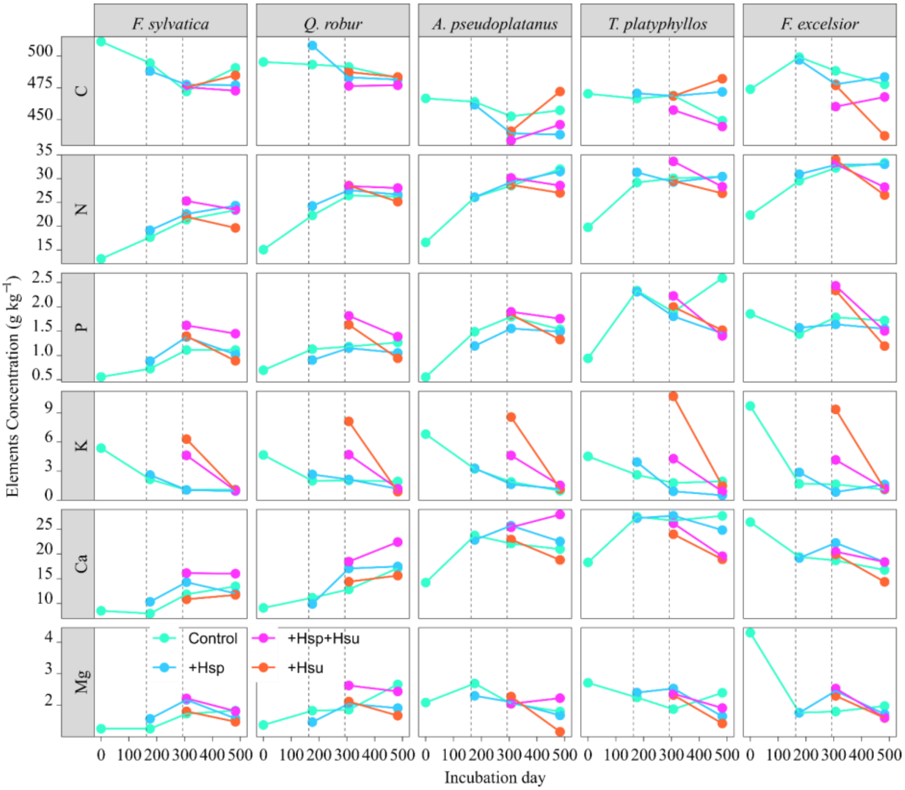

**Figure 5.4** Changes in carbon and nutrient (N, P, K, Ca, and Mg) concentration in decomposing tree litter over time under different treatments of herb litter addition. Values are a combined measurement of three replicates (n=1). Vertical lines show when the spring and/or summer herb litter was added.

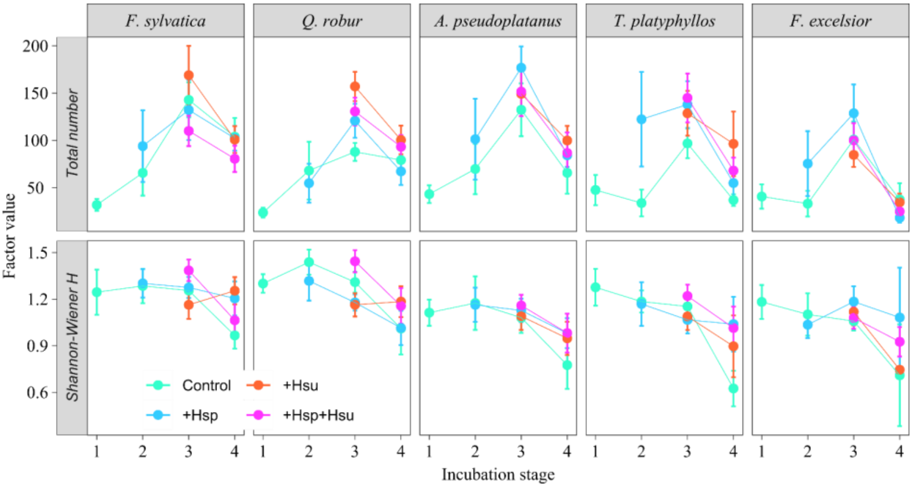

**Figure 5.5** Number of soil fauna, Shannon-Wiener *H*, at four sample dates (Stage 1 = Before herb litter adding; Stage 2 = After spring herb litter adding; Stage 3 = After summer herb litter adding; Stage 4 = After herb litter adding) in five tree litters.

### 5.3.4 Structural equation models

The two SEMs models (Fig. 5.6) showed a good fit to the data, explaining 98% and 90% of the variance in litter decomposition rates in the spring and summer herb litter addition treatments, respectively. The initial quality of tree litter was the largest positive contributor to litter decomposition rates (Fig. 5.6a, b). The remaining litter quality, being strongly positively related to initial litter quality, also showed a positive relationship with decomposition rates. Compared to the addition of spring herb litter (which had a negative effect on tree litter decomposition; Fig. 5.6a), the addition of summer herb litter had a direct positive effect on the decomposition rates and also indirectly affected the decomposition rates of tree litter by increasing nutrient concentrations (Fig. 5.6b). The addition of both spring and summer herb litter had significant positive effects on the abundance of detritivores and omnivores ($p < 0.05$); however, these soil fauna groups showed no significant effect on the decomposition rates of tree litter ($p > 0.05$). The effect of the addition of summer herb litter on the decomposition of tree litter was mainly due to its nutrient replenishment, and not via changes in the abundance of detritivores and omnivores.

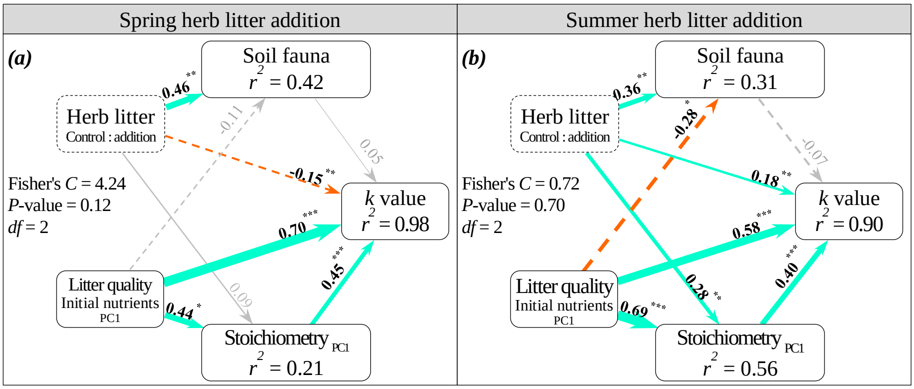

**Figure 5.6.** Direct and indirect effects of litter quality, spring (a) and summer (b) herb litter addition, soil fauna and remaining stoichiometry $_{PC1}$ after addition on the tree litter decomposition rate (*k* value). Continuous and dashed arrows represent positive and negative relationships, respectively. The width of the significance paths has been scaled based on the magnitude of the standardized regression coefficient. Paths with non-significant (ns) effects ($p > 0.05$) were presented with grey arrows. Asterisks *, **, *** show significance levels with *p* values lower than 0.05, 0.01 and 0.001, respectively. Conditional ($R^2c$) $R^2$ values of the fitted linear mixed-effect models are shown below each variable. The variable 'Litter quality' indicates the first component of a PCA (Fig. A5.1, Table A5.5), which is significantly related with initial tree litter C and nutrient (N, P, K, Ca and Mg) concentration (Spearman's $\rho > 0.60$, $p < 0.001$). The 'Stoichiometry (PC1)' indicates the first PCA component that significantly relates to tree litter C and nutrient concentration 14 days after spring or summer herb litter addition. See appendix Table A5.5 and Fig A5.1. 'Soil fauna' indicates the total number of detritivores and omnivores (these two groups of litter fauna are important drivers of tree litter decomposition) at around 40 days after spring or summer herb litter addition, when the soil fauna community structure is relatively stable.

## 5.4 Discussion

The input of herb litter might play a considerable role for carbon and nutrient cycling in forest ecosystems via the leaching of nutrients into the underlying tree litter and altering the quality of food and the habitat for detritivores. In this study, we tested the effects of the addition of seasonal herb litter on tree litter decomposition, tree litter nutrients, and the soil fauna community in tree litter. To the best of our knowledge, this is the first study to experimentally investigate such effects. Partially consistent with our first hypothesis, we demonstrated that the addition of our summer herb litter (*A. podagraria*) positively influenced the decomposition of recalcitrant tree litter. In contrast, the addition of spring herb litter (*A. nemorosa*) and spring + summer herb litter, slightly slowed the decomposition rates of recalcitrant tree litter. In agreement with our second hypothesis, we found an increase in the concentrations of nutrients in tree litter after the addition of summer herb litter. The addition of herb litter showed significant effects on the abundance of soil fauna, especially for omnivores in recalcitrant tree litter, supporting the first part of our third hypothesis. However, the structural equation models showed that there was no indirect effect of herb litter on tree litter decomposition due to an increase in the abundance of soil fauna (detritivores and omnivores), contrary to the second part of our third hypothesis.

### 5.4.1 The effect of the addition of herb litter on the decomposition of tree litter

We observed that the addition of spring herb (*A. nemorosa*) litter inhibited the decomposition of tree litter (except for *F. excelsior* litter), whereas the presence of summer herb (*A. podagraria*) litter accelerated decomposition rates by 10 to 90% compared with the control. Despite the stronger effect on the decomposition of low-quality litter, the mixture effect did not change the order of the decomposition rates of the tree species. The opposite effects of the addition of spring and summer herb litter on the decomposition of tree litter indicates that the decomposition rates of the target litter may not always be stimulated by the addition of high quality litter from a different species (Wu *et al.* 2014; Santonja *et al.* 2017). This may be because of the regulatory function of added litter on N and P concentrations or environmental conditions (Feller *et al.* 2003). The litter of *A. nemorosa* and *A. podagraria* decomposed quickly and had a relatively high initial N and P concentration, respectively (Table 5.1, Fig. A5.1 & A5.3). The N-rich spring herb litter may not alter the N-status in the tree litter or its environment because N-limitation is not likely in a region with high N-deposition such as northern Belgium (Table A5.6) (Bobbink *et al.* 1998). Biodegradation of lignin, such as via white-rot fungus, is thought to be largely restricted by high N input (from both N-rich herb litter and atmospheric N deposition) in the decomposing environment (Thirukkumaran & Parkinson 2000; Koide & Wu 2003; Bonner *et al.* 2019). In contrast, the addition of summer herb litter strongly increased the P concentration of recalcitrant tree litter (e.g., *F. sylvatica* and *Q. robur*) (Fig. 5.4), possibly inducing a positive direct effect on the decomposition rate of tree litter (Fig. 5.6b). This suggests that the effect of the addition of herb litter on the decomposition of tree litter in mixtures might depend on its capacity to alter N- or P-limitation via nutrient transmission from herb to tree litter. Similar patterns have been found in previous studies. For example, the addition of legume (high N-content) litter and N enrichment experiments have shown that higher N input inhibited the decomposition of low quality litter (Ganjegunte *et al.* 2005; Knorr *et al.* 2008), while P enrichment could enhance the decomposition of low quality litter (Feng *et al.* 2018; Ochoa-Hueso *et al.* 2019). The K transfer from P-K-rich herb litter towards underlaying tree litter might increase diversity and activity of microbial decomposers (Kerekes *et al.* 2013; Ochoa-Hueso *et al.* 2019).

Compared to summer herb litter, spring herb contains a higher amount of deterrent compounds (e.g., tannins, which are considered unpalatable for decomposers), but lower hydrophilic compounds (Pérez-Suárez *et al.* 2012). Lukianchuk et al., (2017) reported that *A. nemorosa* contains glycosides, a type of tannins, which are generally considered toxic to some detritivores such as Gastropoda (Mølgaard 1986). On the contrary, summer herb litter makes the tree litter, especially the recalcitrant litter, more palatable for some of the decomposers. For example, we observed a comparatively higher number of Diptera larva in summer herb litter and the underlying tree litter. On the other hand, summer herb litter has a higher water-holding capacity because of the hydrophilic components than spring herb litter, which contributes to moisture retention. The moister microclimate may improve the decay rates of the underlying tree litter (Trogisch *et al.* 2016). Furthermore, the summer herb litter could compensate for the water limitation in recalcitrant litters that dry quickly (e.g. *F. sylvatica* and *Q. robur*) by covering the litter and preventing evaporation.

Mixing the leaf litter from species differing in litter quality changes the physicochemical properties of the decomposition environment as well as the litter itself (Gartner & Cardon 2004). We investigated two plausible explanations for these mixture effects. The first is the movement of substances between the mixed litter types, including the nutrients. The second is the impact on the decomposer community, including its abundance and diversity.

### 5.4.2 The effect of the addition of herb litter on nutrient release

The transfer of nutrients and secondary chemicals from the herb litter to the tree litter might alter its chemical composition. For example, Handa et al. (2014) reported that N could easily be transferred from N-fixing plant litter (high N-rich plant residues) to more recalcitrant litter, influencing the decomposition process (McArthur *et al.* 1994; Schimel & Hättenschwiler 2007). In this study, we observed that the concentration of N, P, K, Ca, and Mg of tree litter after the addition of herb litter was generally higher than the litter in the control treatment. K concentrations were three to five times higher in tree litter when summer herb litter was added (Fig. 5.4). Our results indicate that nutrient flow generally goes from litter with high nutrient content to litter with low nutrient content, which is consistent with the findings from previous studies (Hunter *et al.* 2003; Xu & Hirata 2005; Moore *et al.* 2011). The accumulation of N and P in recalcitrant litter could be attributed to microbial immobilization of mineral forms (Parton *et al.* 2007; Manzoni *et al.* 2008), and to a relatively fast loss of C (via $CO_2$ emission) from the litter (González *et al.* 2014).

### 5.4.3 The effect of the addition of herb litter on soil fauna

Soil fauna play a vital role in ecosystem functioning by regulating C and nutrient release from plant residues (García-Palacios *et al.* 2013). At the start of the incubation period, the abundance of soil fauna was initially higher in labile litter (*T. platyphyllos* and *F. excelsior*) compared with recalcitrant litter (*F. sylvatica* and *Q. robur*); however, this pattern reversed over the course of the experiment (Fig. 5.5). This may be due to the trade-off between litter quality, resulting in fast decomposition and the remaining litter volume acting as a habitat for soil fauna. Litter with a high N-content and soluble carbohydrates, such as in *T. platyphyllos* and *F. excelsior,* is preferentially used as a food source by soil fauna resulting in a higher abundance of soil fauna (Hättenschwiler & Jørgensen 2010). Labile litter decomposes more rapidly than recalcitrant litter; thus, the habitat for soil fauna (litter volume) decreases quickly, resulting in lower fauna abundance (Hättenschwiler *et al.* 2005).

Litter mixture effects are generally attributed to the physical and biochemical changes in the mixture system (Gartner & Cardon 2004). For instance, as known from feeding preferences, mixing plant litter with different characteristics would become progressively more palatable and attract more decomposers (in quantity or diversity) compared with monocultures, resulting in faster decomposition rates than predicted (Hättenschwiler *et al.* 2005; Wu *et al.* 2014; Santonja *et al.* 2017). Additionally, fresh, high-quality leaf litter (e.g., herb litter) could be particularly beneficial for the decomposition of the tree litter underneath when most of the tree litters' nutritional components are already consumed. We found that the addition of high-quality herb litter resulted in a significant increase in the abundance of taxa, but this increase only led to a marginal contribution to tree litter decomposition (Fig.5.6), which may be because the addition of spring herb litter primarily increased the abundance of soil fauna in labile litter, whereas this increase was limited in recalcitrant litter. In contrast, the addition of summer herb litter led to a higher quantitative increase of soil fauna abundance in recalcitrant litter, but the detritivores and omnivores might ingest more herb fragments than tree leaves and mainly use tree litter as habitat (De Deyn *et al.* 2004; Hättenschwiler *et al.* 2005).

Next to soil fauna, soil micro-organisms (bacteria and fungi) are major contributors to the breakdown of leaf litter and their community can be strongly influenced by soil fauna and litter identity (Huhta 2007). Unfortunately, we did not study the soil micro-organism community; however, we think that incorporating the soil micro-organism community into tree–herb litter experiments will be a big step forward in understanding nutrient-cycling in forest ecosystems.

In Summary, consistent with previous work, our study provides evidence that litter quality is the best predictor of decomposition rates (Lummer *et al.* 2012; García-Palacios *et al.* 2016b). However, the presence of high-quality herb litter would alter the accuracy of the prediction. We found that the seasonal addition of herb litter had idiosyncratic effects on tree litter decomposition; *A. nemorosa* showed an inhibitory effect, whereas *A. podagraria* had a stimulatory effect, which could be attributed to litter-specific chemical composition. In addition, summer herb litter extended the habitat of soil fauna leading to higher fauna aggregation in recalcitrant tree litter. In our study, the comparison between soil fauna-mediated paths and nutrient-mediated paths showed that both addition of spring and summer herb litter did not indirectly stimulate decomposition of tree litter via the soil fauna community, in spite of the admixture of herb litter significantly increasing the abundance of soil fauna. The stimulatory effects of summer herb on the decomposition of tree litter were partly attributed to its positive influence on nutrient concentration of tree litter in later stages of decomposition. Considering these contrasting effects of the different understorey herb species, we propose that future experiments should focus on a wider range of herb species to study how their litter characteristics influence litter decomposition and nutrient cycling in forest systems. In addition, the herb community can be very species rich (Landuyt et al. 2019) and therefore follow-up experiments should also investigate the interactive effects when mixing different herb species with tree litter.

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# Appendix

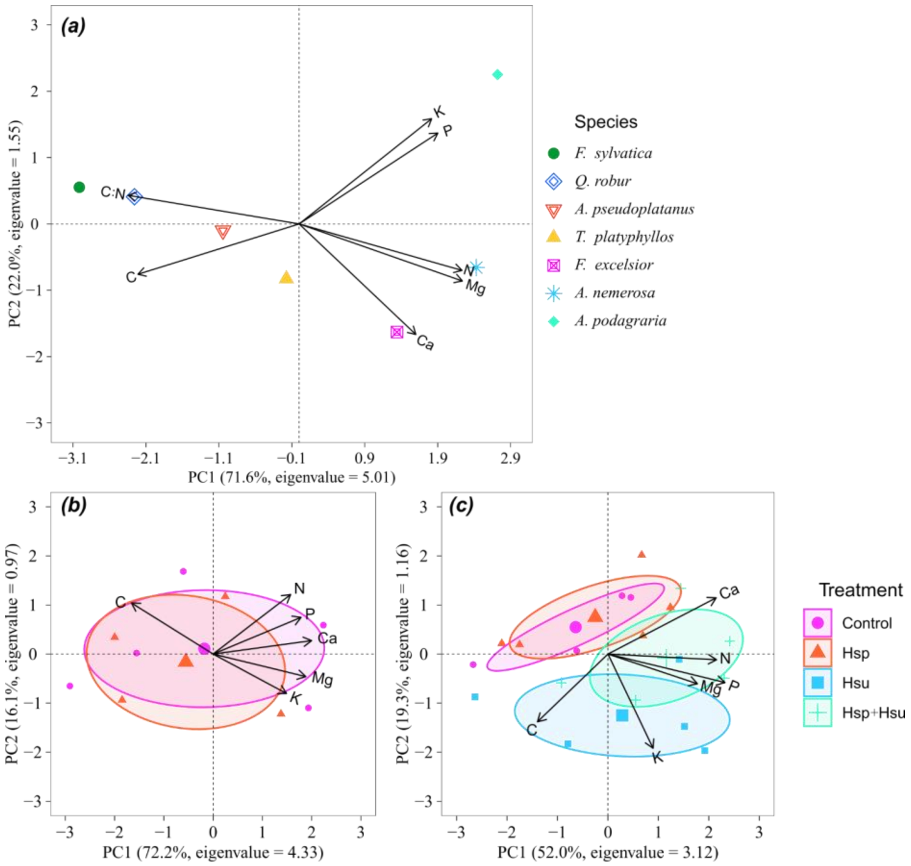


**Figure A5.1**. Principal component analysis of litter quality ((a) initial litter stoichiometry of seven litter species), and litter stoichiometry concentration at 176 and 307 days after (b, spring herb) and (c, summer herb) the litter bag incubation, respectively.

**Table A5.1** Effect of tree species (S), herb treatment (T) and their interaction on decomposition rate (*k*) calculated on the entire decomposition time series. Effects were tested with ANOVA.

| Factor | SS | *df* | *F* | *p* value |
|---|---|---|---|---|
| Species (S) | 39.718 | 4 | 107.9050 | **<0.001** |
| Treatment (T) | 1.334 | 3 | 4.8327 | **0.006** |
| S:T | 3.490 | 12 | 3.1601 | **0.003** |

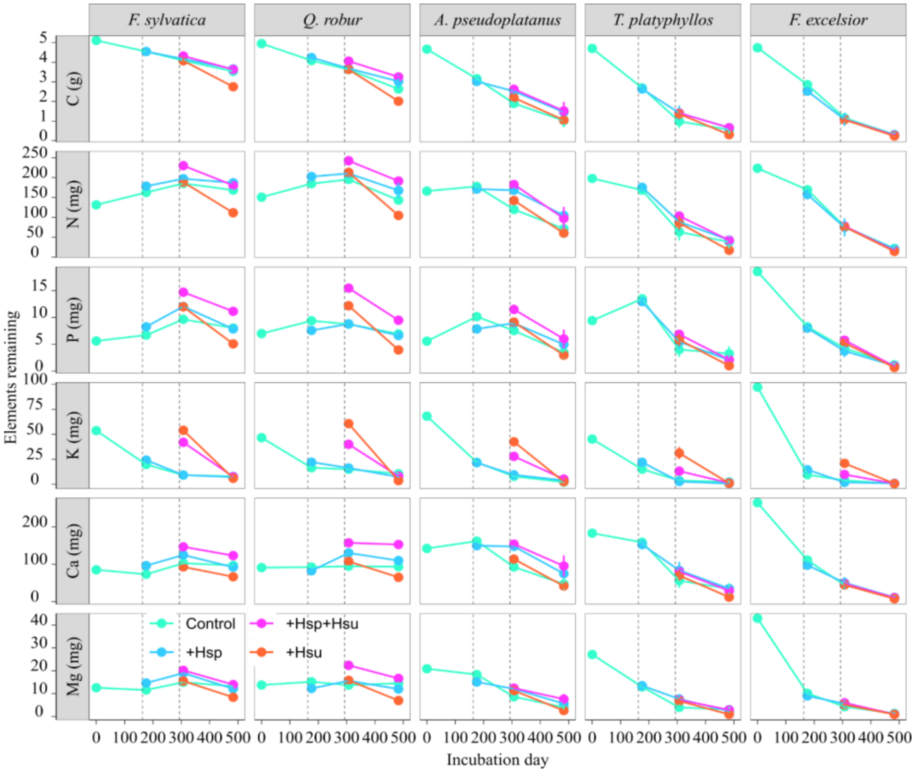


**Figure A5.2** Average carbon (C) and nutrient (N, P, K, Ca, and Mg) stocks in five decomposing tree litter species over time under different litter addition treatments. Control = tree litter only, +Hsp = spring herb litter addition, +Hsu = summer herb litter addition, +Hsp+Hsu = spring and summer herb addition.

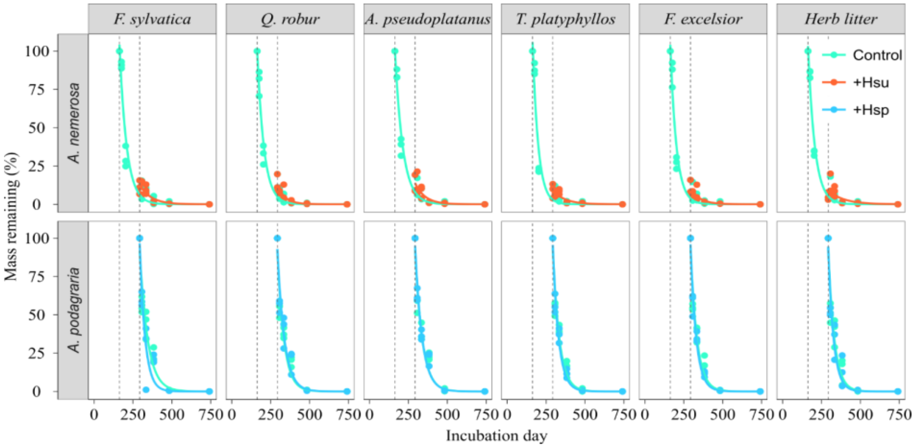


**Figure A5.3** The remaining mass of the spring and summer ephemeral herbaceous litter ('Herb control') and the herb litter above the five species of tree litter (Control = Herb litter without tree litter underneath, *A. nemorosa* = spring herb, +Hsu = the spring under the summer herb, *A. podagraria* = summer herb, +Hsp = the summer herb above the spring herb).

**Table A5.2** Average number of detritivores, omnivores, phytophages, predators, and xylophages in five litter types. (Stage 1 = Before herb litter adding; Stage 2 = After spring herb litter adding; Stage 3 = After summer herb litter adding; Stage 4 = After herb litter adding) in five tree litters).

| Species2 | Stage | Treatment | Detritivores | Omnivorous | Phytophage | Predators | Xylophages |
|---|---|---|---|---|---|---|---|
| *F. sylvatica* | 1 | Control | 2.29±0.24 | 8.73±0.68 | 2.50±0.06 | 2.56±0.25 | |
| | 2 | Control | 5.88±0.56 | 14.43±1.74 | 1.43±0.07 | 1.90±0.13 | 2.00±0.27 |
| | | $+H_{Sp}$ | 6.05±1.37 | 25.92±6.61 | 2.00±0.12 | 3.77±0.95 | |
| | 3 | Control | 7.09±0.91 | 32.35±3.70 | 1.20±0.04 | 1.63±0.09 | 1.00±0.00 |
| | | $+H_{Sp}$ | 10.07±1.87 | 24.85±3.35 | 1.50±0.07 | 1.80±0.14 | 1.25±0.12 |
| | | $+H_{Su}$ | 5.88±0.92 | 35.30±5.80 | | 2.06±0.32 | |
| | | $+H_{Sp}+H_{Sp}$ | 6.11±0.86 | 23.59±4.03 | | 1.69±0.11 | |
| | 4 | Control | 3.78±0.48 | 26.38±3.40 | | 3.67±0.30 | |
| | | $+H_{Sp}$ | 2.93±0.45 | 23.38±4.93 | | 7.80±1.87 | |
| | | $+H_{Su}$ | 3.63±1.09 | 22.36±4.34 | | 4.50±1.06 | |
| | | $+H_{Sp}+H_{Sp}$ | 2.00±0.29 | 21.79±4.06 | | 3.50±0.68 | |
| *Q. robur* | 1 | Control | 1.63±0.07 | 8.24±0.56 | 1.17±0.04 | 1.17±0.04 | |
| | 2 | Control | 6.53±0.83 | 13.04±1.89 | 1.70±0.12 | 1.47±0.10 | 1.00±0.00 |
| | | $+H_{Sp}$ | 4.48±1.02 | 15.83±3.22 | 2.33±0.32 | 1.90±0.15 | 1.50±0.24 |
| | 3 | Control | 6.67±1.01 | 21.61±1.67 | 1.14±0.03 | 1.44±0.06 | 2.00±0.27 |
| | | $+H_{Sp}$ | 8.79±1.65 | 24.58±2.76 | 1.14±0.04 | 1.47±0.12 | 1.33±0.14 |
| | | $+H_{Su}$ | 5.00±0.58 | 34.81±4.48 | 1.00±0.00 | 2.30±0.23 | 1.33±0.17 |
| | | $+H_{Sp}+H_{Sp}$ | 8.15±0.99 | 27.03±4.04 | 1.50±0.11 | 2.00±0.24 | 3.00±1.12 |
| | 4 | Control | 2.18±0.20 | 23.94±3.02 | | 2.22±0.21 | |
| | | $+H_{Sp}$ | 2.31±0.27 | 17.35±4.25 | | 3.43±0.85 | |
| | | $+H_{Su}$ | 4.36±0.70 | 26.84±4.84 | | 2.89±0.57 | |
| | | $+H_{Sp}+H_{Sp}$ | 2.73±0.57 | 20.08±4.73 | | 3.14±0.38 | |
| *A. pseudoplatanus* | 1 | Control | 3.00±0.28 | 13.08±1.23 | 2.00±0.12 | 1.67±0.13 | |
| | 2 | Control | 3.58±0.32 | 18.44±2.16 | 1.60±0.08 | 1.93±0.14 | |
| | | $+H_{Sp}$ | 7.39±1.45 | 37.20±6.99 | 1.17±0.06 | 1.67±0.16 | 2.67±0.96 |
| | 3 | Control | 8.18±1.21 | 31.16±2.80 | 1.20±0.04 | 2.05±0.13 | |
| | | $+H_{Sp}$ | 10.18±2.16 | 30.84±3.61 | 1.63±0.15 | 2.11±0.23 | |
| | | $+H_{Su}$ | 4.97±0.57 | 42.48±5.56 | | 1.72±0.13 | 1.33±0.17 |
| | | $+H_{Sp}+H_{Sp}$ | 5.74±1.07 | 32.97±5.54 | | 3.82±0.38 | |
| | 4 | Control | 3.80±0.60 | 21.35±2.69 | | 1.67±0.12 | |
| | | $+H_{Sp}$ | 3.45±0.74 | 23.26±4.58 | | 3.00±0.36 | |
| | | $+H_{Su}$ | 2.13±0.22 | 25.95±5.26 | | 2.75±0.39 | |
| | | $+H_{Sp}+H_{Sp}$ | 1.75±0.18 | 27.06±4.92 | | 4.75±0.96 | |
| *T. platyphyllos* | 1 | Control | 2.33±0.17 | 15.96±1.84 | 1.00±0.00 | 2.67±0.19 | |
| | 2 | Control | 2.22±0.18 | 14.47±1.61 | 1.22±0.04 | 2.08±0.14 | |
| | | $+H_{Sp}$ | 3.20±0.34 | 38.35±8.92 | 1.43±0.12 | 2.50±0.53 | |
| | 3 | Control | 11.81±2.60 | 22.38±2.34 | 1.20±0.04 | 1.93±0.12 | |
| | | $+H_{Sp}$ | 11.09±1.98 | 26.76±3.39 | 1.33±0.06 | 2.20±0.20 | |
| | | $+H_{Su}$ | 7.22±1.40 | 3.00±3.92 | | 1.92±0.20 | |
| | | $+H_{Sp}+H_{Sp}$ | 6.21±1.54 | 33.91±5.39 | | 2.92±0.35 | |
| | 4 | Control | 1.50±0.09 | 14.57±1.53 | | 1.67±0.14 | |
| | | $+H_{Sp}$ | 1.60±0.12 | 18.50±4.30 | | 2.00±0.33 | |
| | | $+H_{Su}$ | 2.62±0.64 | 28.78±5.91 | 1.50±0.13 | 2.67±0.42 | |
| | | $+H_{Sp}+H_{Sp}$ | 2.00±0.33 | 17.50±3.56 | | 5.29±0.31 | |
| *F. excelsior* | 1 | Control | 4.75±0.37 | 11.83±1.22 | | | |
| | 2 | Control | 3.31±0.27 | 12.69±1.48 | 1.50±0.05 | 1.60±0.09 | |
| | | $+H_{Sp}$ | 3.83±0.77 | 23.56±5.68 | | 2.10±0.20 | |
| | 3 | Control | 9.20±1.52 | 21.99±1.91 | | 1.58±0.10 | |
| | | $+H_{Sp}$ | 6.38±0.88 | 28.04±3.65 | 1.67±0.11 | 1.85±0.11 | |
| | | $+H_{Su}$ | 4.50±0.82 | 24.59±3.05 | | 2.00±0.15 | 1.50±0.20 |
| | | $+H_{Sp}+H_{Sp}$ | 4.17±0.60 | 26.69±4.31 | | 3.71±0.38 | |
| | 4 | Control | 3.25±0.18 | 31.33±4.33 | | 1.25±0.06 | |
| | | $+H_{Sp}$ | 2.29±0.37 | 6.40±1.65 | | 1.75±0.20 | |
| | | $+H_{Su}$ | 1.50±0.17 | 18.40±3.27 | | 1.67±0.12 | |
| | | $+H_{Sp}+H_{Sp}$ | 1.75±0.08 | 11.20±2.23 | | 3.00±0.37 | |

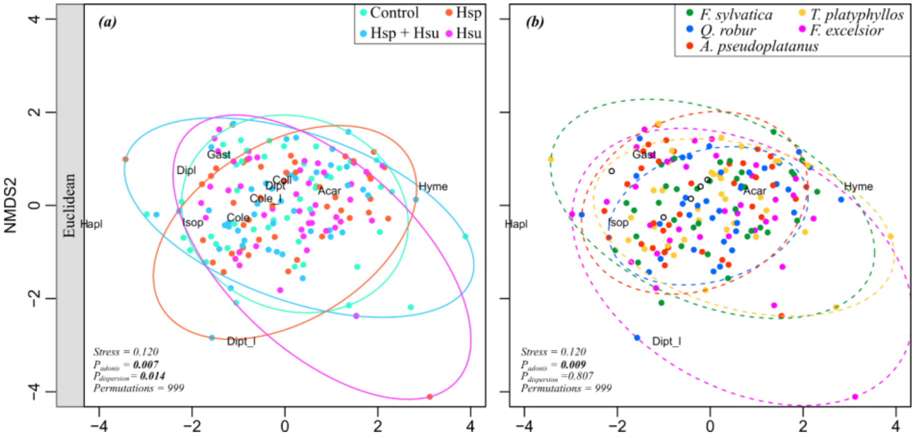


**Figure A5.4** Nonmetric multidimensional scaling (NMDS) of detritivore and omnivore communities under different levels of herb litter additions (a) and according to tree litter types (b). Distances among communities were measured as *Euclidian* (a, b). Control = tree litter only, +Hsp = spring herb litter addition, +Hsu = summer herb litter addition, +Hsp+Hsu = spring and summer herb litter addition. Significances of the compositional differences between the tree species and herb litter addition treatments and of the compositional variation within those treatments are provided as $P_{adonis}$ and $P_{dispersion}$, respectively. The envelopes represent 95% confidence of the mean sample score of the treatments and tree species types. (Detritivores: Dipl = Diplopoda, Dipt = Diptera, Dipt_l = Diptera larva, Gast = Gastropoda, Hapl = Haplotaxida, Isop = Isopoda; Omnivorous: Acar = Acari, Cole = Coleoptera, Cole_l = Coleoptera larva, Coll = Collembola, Derm=Dermaptera, Hyme = Hymenoptera.)

**Table A5.3** Effect of tree species, litter treatment and their interaction on the total number of individuals and Shannon-Wiener *H* diversity index of soil fauna communities found in the tree litter. Effects were tested with ANOVA.

| Factor | Df | Total number of individuals | Shannon-Wiener H |
|---|---|---|---|
| Species (S) | 4 | **0.004** | **< 0.001** |
| Treatment (T) | 3 | **< 0.001** | 0.301 |
| S:T | 12 | 0.966 | 0.987 |

**Table A5.4** Results from two-way ANOVA with total number of individuals, number of detritivores and omnivores, and Shannon-Wiener *H* as response variables, and tree species and herb treatment as explanatory variables. Significant *P* values are highlighted in bold.

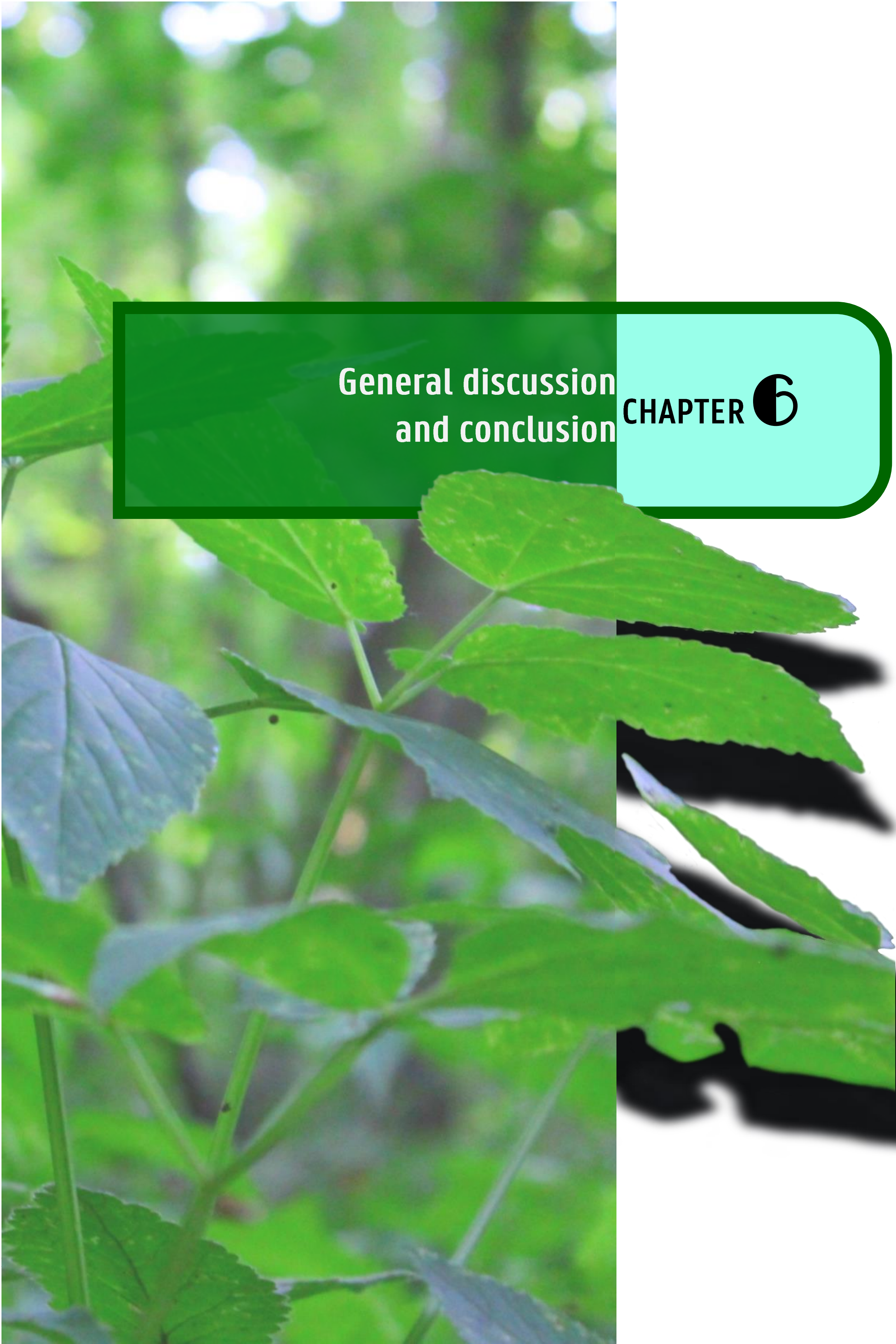
| Index | Factor | *Df* | Sum-Sq | Mean-Sq | *F* | *p* |
|---|---|---|---|---|---|---|
| Total number of individuals | Species (S) | 4 | 2.970 | 0.742 | 3.983 | **0.004** |
| | Treatment (T) | 3 | 9.720 | 3.240 | 17.403 | < **0.001** |
| | S:T | 12 | 0.960 | 0.080 | 0.430 | 0.952 |
| | Residuals | 402 | 74.850 | 0.186 | | |
| Number of detritivores and omnivorous | Species (S) | 4 | 2.680 | 0.669 | 2.953 | **0.020** |
| | Treatment (T) | 3 | 10.660 | 3.553 | 15.675 | < **0.001** |
| | S:T | 12 | 1.070 | 0.089 | 0.393 | 0.966 |
| | Residuals | 402 | 91.120 | 0.227 | | |
| Shannon-Wiener *H* | Species (S) | 4 | 3.060 | 0.766 | 7.204 | < **0.001** |
| | Treatment (T) | 3 | 0.390 | 0.130 | 1.223 | 0.301 |
| | S:T | 12 | 0.400 | 0.034 | 0.316 | 0.987 |
| | Residuals | 402 | 42.750 | 0.106 | | |

**Table A5.5** Pearson correlations between the litter traits (the initial traits, and the nutrient traits 14 days after spring and summer addition, respectively) and the component 1 and 2 of the litter nutrients PCA (Fig. A5.4). Asterisks shows significance level: $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

| | Initial quality | | 2 weeks after spring herb litter addition | | 2 weeks after summer herb litter addition | |
|---|---|---|---|---|---|---|
| | PC1 | PC2 | Nutrient remaining PC1 | Nutrient remaining PC2 | Nutrient remaining PC1 | Nutrient remaining PC2 |
| TC | $-0.76^{***}$ | $-0.59^{***}$ | $-0.81^{***}$ | $0.51^{**}$ | $-0.54^{***}$ | $-0.54^{***}$ |
| TN | $0.93^{***}$ | -0.05 | $0.77^{***}$ | $0.59^{***}$ | $0.84^{***}$ | -0.05 |
| TP | $0.90^{***}$ | $-0.39^{*}$ | $0.87^{***}$ | $0.37^{*}$ | $0.90^{***}$ | $-0.23^{*}$ |
| TK | $0.83^{*}$ | $-0.31^{*}$ | $0.73^{***}$ | $-0.39^{*}$ | $0.35^{**}$ | $-0.75^{***}$ |
| TCa | $0.63^{***}$ | $0.56^{**}$ | $0.92^{***}$ | -0.23 | $0.69^{***}$ | $0.24^{*}$ |
| TMg | $0.57^{***}$ | $0.57^{***}$ | $0.98^{***}$ | 0.13 | $0.84^{***}$ | $0.45^{***}$ |

**Table A5.6** Location and litter N concentration and C:N ratio of five typical tree species in European temperate deciduous forests, and respective N deposition recorded in the closest region (these data were reported by Maes *et al.* 2019).

| Location | | *Tree species* | N (%) | C:N | N deposition (kg ha$^{-1}$yr$^{-1}$) | Reference |
|---|---|---|---|---|---|---|
| Belgium | Gontrode | *F. sylvatica* | 1.31 | 38.92 | 21.60 | This thesis |
| Belgium | Zedelgem | *F. sylvatica* | 1.63 | 30.40 | 31.00 | (Setiawan *et al.* 2016; Rahman *et al.* 2018) |
| Denmark | Central | *F. sylvatica* | 1.30 | 42.00 | 19.00 | (Ritter & Vesterdal 2006; Schelfhout *et al.* 2017) |
| France | Haute-Normandie | *F. sylvatica* | 1.35 | 36.79 | 15.10 | (Aubert *et al.* 2010) |
| Germany | Thuringia | *F. sylvatica* | 0.83 | 56.06 | 17.10 | (Jacob *et al.* 2009; Langenbruch *et al.* 2012) |
| Germany | Lower Saxony | *F. sylvatica* | 0.78 | 57.50 | 17.10 | (Yang *et al.* 2019) |
| Germany | Thuringia | *F. sylvatica* | 0.83 | 56.06 | 17.10 | (Langenbruch *et al.* 2012; Cesarz *et al.* 2016) |
| Germany | Thuringia | *F. sylvatica* | 0.88 | 59.00 | 17.10 | (Langenbruch *et al.* 2012; Scheibe *et al.* 2015) |
| Poland | Lignite Mine | *F. sylvatica* | 0.74 | 59.82 | 17.20 | (Horodecki *et al.* 2019) |
| Poland | Biadaszki | *F. sylvatica* | 0.84 | 55.90 | 17.20 | (Hobbie *et al.* 2006) |
| Poland | Central | *F. sylvatica* | 0.74 | 59.82 | 17.20 | (Horodecki & Jagodziński 2017) |
| Belgium | Gontrode | *Q. robur* | 1.51 | 32.87 | 21.60 | This thesis |
| Belgium | Zedelgem | *Q. robur* | 1.62 | 30.50 | 31 | (Setiawan *et al.* 2016; Rahman *et al.* 2018) |
| Denmark | Central | *Q. robur* | 1.60 | 32.00 | 19.00 | (Schelfhout *et al.* 2017) |
| Poland | Lignite Mine | *Q. robur* | 1.04 | 44.47 | 17.20 | (Horodecki *et al.* 2019) |
| Poland | Lignite Mine | *Q. robur* | 0.77 | 60.20 | 17.20 | (Horodecki *et al.* 2019) |
| Poland | Southern | *Q. robur* | 0.61 | 74.10 | 17.20 | (Stanek *et al.* 2020) |
| Poland | Biadaszki | *Q. robur* | 0.71 | 68.70 | 17.20 | (Hobbie *et al.* 2006) |
| Poland | Central | *Q. robur* | 0.77 | 60.20 | 17.20 | (Horodecki & Jagodziński 2017) |
| Belgium | Gontrode | *A. pseudoplatanus* | 1.66 | 28.10 | 21.60 | This thesis |
| Czech Republic | Prague | *A. pseudoplatanus* | 1.30 | 31.40 | 15.80 | (Špaldoňová & Frouz 2014) |
| Denmark | Central | *A. pseudoplatanus* | 1.70 | 27.00 | 19.00 | (Schelfhout *et al.* 2017) |
| Germany | Thuringia | *A. pseudoplatanus* | 1.17 | 36.40 | 17.10 | (Jacob *et al.* 2009) |
| Germany | Lower Saxony | *A. pseudoplatanus* | 1.11 | 36.60 | 17.10 | (Yang *et al.* 2019) |
| Germany | Thuringia | *A. pseudoplatanus* | 1.17 | 36.40 | 17.10 | (Cesarz *et al.* 2016) |
| Germany | Thuringia | *A. pseudoplatanus* | 1.06 | 46.00 | 17.10 | (Scheibe *et al.* 2015) |
| Poland | Lignite Mine | *A. pseudoplatanus* | 1.10 | 40.25 | 17.20 | (Horodecki *et al.* 2019) |
| Poland | Biadaszki | *A. pseudoplatanus* | 0.94 | 49.10 | 17.20 | (Hobbie *et al.* 2006) |
| Poland | Central | *A. pseudoplatanus* | 1.10 | 40.25 | 17.20 | (Horodecki & Jagodziński 2017) |
| UK | Sheffield | *A. pseudoplatanus* | 2.20 | | 14.10 | (Cornelissen *et al.* 2000) |
| UK | Preston | *A. pseudoplatanus* | 1.45 | 33.00 | 14.10 | (Rajapaksha *et al.* 2013b, a) |
| UK | Ceredigion | *A. pseudoplatanus* | 1.20 | 38.01 | 14.10 | (Jones *et al.* 2019) |
| Belgium | Gontrode | *T. platyphyllos* | 1.98 | 23.78 | 21.60 | This thesis |
| Belgium | Zedelgem | *T. cordata* | 2.25 | 21.70 | 31 | (Setiawan *et al.* 2016) |
| Denmark | Central | *T. cordata* | 1.80 | 28.00 | 19.00 | (Schelfhout *et al.* 2017) |
| Germany | Thuringia | *T. spp.* | 1.19 | 37.93 | 17.10 | (Jacob *et al.* 2009; Langenbruch *et al.* 2012) |
| Germany | Lower Saxony | *T. cordata* | 1.12 | 40.50 | 17.10 | (Yang *et al.* 2019) |
| Germany | Thuringia | *T. spp.* | 1.19 | 37.93 | 17.10 | (Langenbruch *et al.* 2012; Cesarz *et al.* 2016) |
| Germany | Thuringia | *T. cordata* | 1.40 | 35.00 | 17.10 | (Scheibe *et al.* 2015) |
| Poland | Biadaszki | *T. cordata* | 1.22 | 37.00 | 17.20 | (Hobbie *et al.* 2006) |
| Belgium | Gontrode | *F. excelsior* | 2.23 | 21.22 | 21.60 | This thesis |
| Denmark | Central | *F. excelsior* | 1.90 | 25.00 | 19.00 | (Schelfhout *et al.* 2017) |
| Germany | Thuringia | *F. excelsior* | 1.57 | 27.60 | 17.10 | (Jacob *et al.* 2009; Langenbruch *et al.* 2012) |
| Germany | Lower Saxony | *F. excelsior* | 1.03 | 40.20 | 17.10 | (Yang *et al.* 2019) |
| Germany | Thuringia | *F. excelsior* | 1.57 | 27.60 | 17.10 | (Langenbruch *et al.* 2012; Cesarz *et al.* 2016) |
| Germany | Thuringia | *F. excelsior* | 1.22 | 40.00 | 17.10 | (Langenbruch *et al.* 2012; Scheibe *et al.* 2015) |
| Poland | Lignite Mine | *F. excelsior* | 1.12 | 39.41 | 17.20 | (Horodecki *et al.* 2019) |
| UK | Preston | *F. excelsior* | 1.51 | 31.30 | 14.10 | (Rajapaksha *et al.* 2013b, a) |
| UK | Ceredigion | *F. excelsior* | 2.14 | 20.80 | 14.10 | (Jones *et al.* 2019) |



## CHAPTER 6

# General discussion and conclusion

The functional role of the forest overstorey layer, that is, its influence on key ecological processes in forest ecosystems, has been extensively studied (Dayton 1975; Del Tredici 2001; Barbier *et al.* 2008), e.g., microclimate (Royer *et al.* 2011; Zellweger *et al.* 2020), C and nutrient cycling (Prescott 2002; Leppert *et al.* 2017), understorey biotic communities (Mitchell *et al.* 2012; Chen *et al.* 2018), and soil properties (Boča *et al.* 2014). More specifically, as a major source for C and nutrient, the litter turnover processes of overstorey tree species (both monocultures and mixtures) has been widely studied for decades (Attiwill 1968; Berg 2000; Wu *et al.* 2014). However, the role of understorey herbs (as an indispensable forest component) has been largely overlooked in the study of litter decomposition, until relatively recently (Chomel *et al.* 2016; He *et al.* 2020a). The herb species in the understorey return high-quality residues to the forest floor, with inputs spread across the vegetation season, due to broad between-species differences in phenology. For example, *A. nemorosa*, *Ficaria verna, and Corydalis cava* generally senesce in late spring, while *A. podagraria and Galeobdolon luteum* generally senesce in late autumn (Jagodziński *et al.* 2016). The work in this thesis started from the expectation that the herb layer in forest ecosystems affects C and nutrient turnover processes either directly, through its nutrient release during decomposition (Cornelissen & Thompson 1997) leading to mixture effects (Chomel *et al.* 2016; He *et al.* 2020a), or indirectly, through its regulatory effects on microclimate (Gilliam 2007; Matsushima & Chang 2007; Landuyt *et al.* 2019). Nonetheless, knowledge on the functional role of the understorey layer for litter decomposition processes is still limited, it is particularly limited in the context of global change.

The major aims of this thesis were (i) to assess the regulatory function of the understorey layer for soil microclimate and decomposition processes in soil, through its canopy thermal and hydrological buffering, under both natural condition and in the context of environmental changes, and (ii) to determine the mixture function of herbaceous litter addition on humidity, nutrient dynamics, detritivore performance, and soil fauna community composition in litter layer, then provides mixture effects on decomposition of tree litter species. Different experimental study approaches, including forest understorey removal (**Chapter 2**), mesocosms (**Chapter 3**), laboratory microcosms (**Chapter 4**), and *in-situ* litter incubation experiments (**Chapter 5**), were conducted to obtain the regulatory function and mixture function of the understorey layer on litter decomposition. In this concluding chapter, a general overview of the most relevant results of the four previous chapters is discussed, together with some limitations of the study, designs and implications for forest management, thereby suggesting directions for future research.

## 6.1 Regulatory function of the understorey layer

### 6.1.1 Regulation of the soil micro-environment

The understorey layer in forest ecosystems is expected to have similar functions for the soil microclimate as the overstorey layer does, as it has similar canopy structure to buffer against the ambient macroclimate (Wang *et al.* 2014; De Long *et al.* 2016; Özkan & Gökbulak 2017). The near-surface soil microclimate (including temperature and moisture) is expected to vary in response to the presence of the understorey layer (alive) and herb litter (senescence). We measured functional traits of the understorey layer and its litter production on the near-ground microclimate, by comparing the microclimate condition when an understorey layer (or its litter) was present versus when it was absent. Measurements included the daily thermal buffering (difference between daily maximum and minimum temperature at 5 cm depth), percent volumetric soil water content (top soil layer, in 0-5 cm depth), forest non-woody debris moisture content (by collecting litter in 20 *20 cm plot), and moisture content of tree litters.

In line with our expectations, our data demonstrate that the herbaceous canopy could significantly buffer thermal (both hot and cold) extremes (Fig. 6.1 a), while the thermal buffering decreased with incubation day, as the dominant ephemeral species, *A. nemorosa*, was withering over time (**Chapter 2**). The soil temperature increase was subtle under understorey removal treatments and mostly appeared in the first 30 days of incubation (**Chapter 2**). This was much lower than other understorey removal studies, which reported that the understorey removal increased soil temperature by 0.5°C to 1.7 °C (Matsushima & Chang 2007; Zhao *et al.* 2013; Wang *et al.* 2014; Özkan & Gökbulak 2017). There are a few reasons for the lower temperature increase. First our temperature monitoring started in early April and ended in July, which was a rainy period in Belgium. The rainfall might cool down the soil in both herb presence and absence plots. Second, the dominant understorey species is *A. nemorosa*, with an average height of only 14 cm, which made the understorey canopy comparable thinner than at *Calamagrostis canadensis* dominated boreal forests (Matsushima & Chang 2007) and *Dicranopteris* spp. dominated subtropical forest (Wang *et al.* 2014), resulting in a lower temperature buffering. Finally, the short-term period of monitoring probably also influenced the result of temperature buffering.

Besides the temperature buffering, the understorey layer also showed remarkable effects on the moisture distribution near the soil surface, by maintaining water in the litter layer, through reducing the water evaporation (Fig. 6.1 c) and increasing the litter water maintaining capacity (Fig. 6.1 d). The understorey canopy, on the one hand, reduced water evaporation by preventing solar radiation from penetrating into the forest floor during the growing season (**Chapter 2**) and, on the other hand, increased water retention capacity by producing high hydrophilic litter in the senescence season (**Chapter 4**). Thus, it may create a favorable (more humid) environment for litter decomposition, as the moisture content is one of the key factors driving soil biotic activity in litter decomposition (Wang *et al.* 2019). Compared to the litter layer, the presence of understorey layer, on the contrary, actually reduced soil water content (Fig 6.1 b) through its rainfall interception and its leaves transpiration. This is in accordance with results obtained by, e.g., Clinton (2003), Xiong *et al.* (2008), and Özkan & Gökbulak (2017) in different biomes, where soil moisture in the topsoil decreased as the understorey layer impeded water input and drained water through roots in the topsoil, and this transpiration generally starts in April when the herb layer starts sprouting from the soil. Nonetheless, our study may not be fully representative for longer-term water availability patterns (like a full year or a long-term study), or for different biomes, as some long-term studies and studies in different forest types showed that presence of understorey layer actually increased soil moisture (Zhao *et al.* 2013; Wang *et al.* 2014).

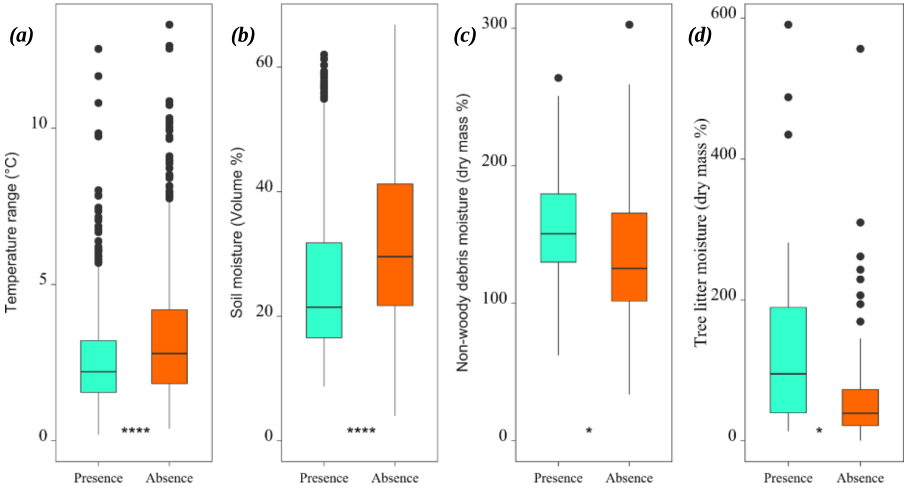


**Figure 6.1.** Effect of understorey vegetation on soil temperature range (a), soil moisture (b), forest non-woody debris moisture content (c), and the effect of herb litter on moisture content of tree litter (d). Asterisks *, **** show significance levels with $p$ values lower than 0.05 and 0.0001, respectively.

The influence of the understorey layer on the near-surface microclimate was also related to the total cover of understorey vegetation. Higher understorey cover generally leads to higher solar radiation reflection and rainfall interception compared to low understorey cover (Vanneste *et al.* 2020). Thus, the amount of light and rainfall reaching the forest floor is largely governed by the overall density (here measured as cover) of the understorey canopy. Soil temperature buffering in plots with high understorey cover (cover > 90%) was 0.69 ± 0.08°C higher than in the plots with relatively lower cover (cover < 90%). This understorey cover effect on soil moisture buffering was subtle (-0.68±1.02%) (**Chapter 2**). Based on the colder and drier soil microclimate under the high understorey cover, we assumed that the ecological processes under the herb cover would be inhibited, and the effects of environmental changes on belowground biotic and abiotic processes would be also suppressed, the understorey layer is expected to have positive responses to warming and tree canopy management (De Frenne *et al.* 2015; Blondeel *et al.* 2019).

### 6.1.2 Regulation of the soil fauna community

Isopoda (woodlice) and Diplopoda (millipedes) are two important taxa of macro-detritivores, feeding on dead plant material in temperate forests (De Smedt *et al.* 2018). As two different biotypes, isopoda and diplopoda show different habitat preference. The isopoda are mostly sensitive to cold and drought, while diplopoda are generally more drought tolerant. To avoid the cold environment, isopods prefer to migrate according to environmental temperature, whereas the diplopoda are generally active at the *ectone* between the soil and litter layer, as their body is mostly covered with a hard and dense exoskeleton (De Smedt *et al.* 2018). We found that the abundance of isopoda was significantly higher in the plots without an understorey layer ($F = 5.69$, $p = 0.018$, Fig. 6.2 a), while the abundance of diplopoda was slightly lower ($p > 0.05$) (**Chapter 2**).

We observed different responses of isopoda and diplopoda to the *A. podagraria* litter addition in both laboratory and *in-situ* conditions, despite both relying on forest litter and both having preference for high-quality litter (David & Handa 2010; Fontana *et al.* 2019). Under laboratory conditions, the feeding resources were mandated, that is, the detritivores could either ingest the provided monoculture tree litter or admixture tree and herb litter, or suffer from starvation. The *A. podagraria* litter addition significantly enhanced assimilation (here expressed as weight gain) of isopods ($F = 5.56$, $p = 0.02$, Fig. 6.2 c), and reduced dissimilation (here expressed as weight loss) of diplopoda. This implies that the addition of herb litter promoted their appetite and decelerated weight loss than feeding on monospecific tree litters, despite the observation that herb litter led to 38.33±8.76% higher mortality of diplopoda compared to litter monocultures ($F = 19.15$, $p < 0.001$, Fig. 6.2 b) (and also see **Chapter 4**).

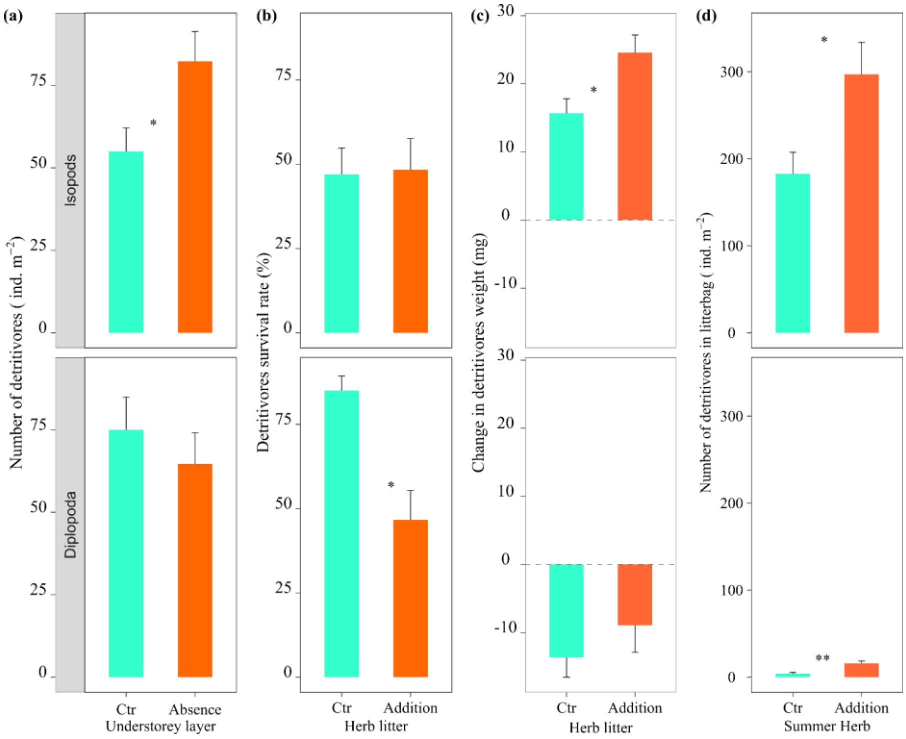


**Figure 6.2.** Effects of understorey layer and understorey litter on performance of Isopoda and Diplopoda. (a) shows the response of detritivores to understorey removal (**Chapter 2**, understorey presence *vs*. absence. Detritivore taxa Isopods and Diplopoda were extracted from forest non-woody debris litter collected at 20 by 20 cm plots, unpublished data), (b) and (c), respectively, shows the survival rate and weight change of detritivores in the tree litter with or without herb litter (*A. podagraria*) addition (**Chapter 4**, laboratory microcosms, the data is for *O. asellus* (Isopoda) and *G. marginata* (Diplopoda)), and (d) shows the abundance of detritivores in litter bag with and without summer herb (*A. podagraria*) litter addition, above and below the dividing line represents the mean number of detritivores extracted from herb litter and tree litter, respectively (**Chapter 5**, in-suit litter bag incubation, soil fauna were extracted from litter bags). 'Ctr' represents Control (without herb layer removal or without herbaceous litter addition). Asterisks *, **** show significance levels with $p$ values lower than 0.05 and 0.0001, respectively.

In the field, the detritivores are free to choose their feeding materials and resting places. The *A. podagraria* litter addition was attractive for detritivores and increased the total number of both isopods ($F$ = 4.347, $p$ = 0.039) and diplopoda ($F$ = 8.219, $p$ = 0.00482). This indicated that the additional *A. podagraria* litter is at least not toxic for both types of detritivores. Therefore, the high mortality of diplopoda in the lab experiment is not likely to be attributed to inhibitory components in *A. podagraria* litter. A possible explanation is that the living environment of the diplopoda (drought tolerant species) in mixture litter was more humid than in the monocultures, which may benefit pathogenic organisms (e.g., a parasitic fungus, *Beauveria bassiana*) and led to an increase in mortality (Bastow *et al.* 2008). Consequently, we found that the mortality of diplopoda was related to the litter moisture content. On the other hand, adding *A. podagraria* litter seems to benefit isopods, as mortality of isopods did not decrease by adding herb litter. They gained weight in the mixtures and the number of isopods increased significantly in *A. podagraria* litter addition in the field ($F = 4.347$, $p = 0.039$, Fig. 6.2 d), and about one third of them were extracted from the herb litter.

### 6.1.3 Regulation of tree litter decomposition

The understorey layer is expected to create a cooler and drier soil microenvironment (§6.1.1 and 6.1.2), which is generally less favorable for litter decomposition (Wang *et al.* 2014; De Long *et al.* 2016; Özkan & Gökbulak 2017). In contrast with previous studies, we observed that there was no difference in litter mass loss between the presence and absence of an understorey layer, neither in high-quality litter nor in low-quality litter (**Chapter 2**). The main reason for this non-significant result could be that the dominant herb species is rather ephemeral (only forming aboveground biomass for about three months), thus, the factors that related to litter decomposition may not have changed sufficiently in this short time period by the understorey removal. Alternatively, despite the soil microenvironment being colder and drier under the understorey than under understorey removal, the ecological contrast might not have been sufficiently large to be a limit factor for litter decomposition. For example, understorey removal increased soil moisture content, whereas it did not alter litter moisture content of two types of litter that were buried in belowground (**Chapter 2**). On the other hand, in contrast to long-term studies (Fanin *et al.* 2019; He *et al.* 2020b), the microbial decomposer communities did not change immediately after the understorey removal, as threatening climate events, e.g., hot and drought weather, for decomposer communities generally appear in summer.

Cover and biomass productivity of understorey plants express soil physico-chemical properties (Zhou *et al.* 2016) and the understorey cover will, in turn, influences the microbial communities and litter decomposition processes in the soil, by regulating the soil microclimate (Fanin *et al.* 2019; He *et al.* 2020b). The mass loss of both green tea and rooibos tea were negatively correlated with the understorey cover in different types of soil, under natural conditions and under the environmental enrichment treatments

(**Chapter 2 and 3**). The understorey vegetation communities in warming and more light receiving illimitation environments were reported to have a higher leaf area index or percentage vegetation cover (Blondeel et al. 2020; Figure 3.5), which imply more a higher nutrients and water supplying from the soil just to acquire their nutrients requirements. These denser herb canopy structure would intercept more rainfall, evaporate more soil water and shade more solar radiation, which then leading leads to further soil drying and cooling of the soil. The lager amount of nutrients uptake taken up would, on the other hand, intensify the nutrient competition between plants and competition between microbial decomposers, particularly in nutrient poor soil (Fig. 3.5). In this competition, compared to the understorey vegetation, the soil microbial communities generally being at the disadvantage (Hättenschwiler & Vitousek 2000; Matsushima & Chang 2007; Kaneda *et al.* 2020). Consequently, high understorey cover and production involve more nutrient and water uptake, possibly leading to nutrient, water, or even thermal stress for microbial decomposers in the soil.

## 6.2 Litter mixture effects

### 6.2.1 Litter mixture effects of herb litter on microenvironment

The mixture effects are partly attributed to the physical improvement of the microenvironmental conditions (mostly litter moisture content) in their co-occurring system which is expected to be more favorable for decomposers (Makkonen *et al.* 2013). Litter from understorey vegetation generally has higher water retention capacity than overstorey tree species, due to its physical characteristics. For example, the species in the Apiaceae, Asteraceae and Poaceae family generally have a low surface-to-volume ratio resulting in a high water-holding capacity (Makkonen *et al.* 2013). On the other hand, due to its chemical components, for example, species in Apiaceae, Fabaceae and Cornaceae family generally contain high hydrophilic substances which can lock water in the litter (Liu *et al.* 2013). As a species in the Apiaceae family, *A. podagaria* litter, enriched litter moisture content and stimulated decomposition of tree litter as expected (**Chapter 4, 5**). However, the herb litter-driven mixture effects are unlikely to be attributed to the improvement of micro-environmental conditions, as we also observed that the moisture content in the tree litter layer under the “below addition of herb litter” (**Chapter 4**) was much higher than it was in the above addition, whereas the decomposition of three litter did not enhance in the wetter environment. This contrasts to the previously established improved microenvironmental conditions theory (Wardle *et al.* 2003;

Makkonen *et al.* 2013; Joly *et al.* 2017). Furthermore, the more humid decomposing environment did not delight to all decomposers, on the contrary, it led to weight loss and high mortality of the millipede *G. marginata*, a species favoring dryer conditions (**Chapter 4**). The higher litter moisture content might also intensify the oxygen stress in the litter layer (Kooijman & Cammeraat 2010; Smith *et al.* 2015; Sierra *et al.* 2017), resulting in the decrease in population of decomposers. The below addition of herb litter led to 200% of litter moisture content in litter layer (**Chapter 4**).

### 6.2.2 Litter mixture effects of herb litter on soil fauna communities

In correspondence with previous research (Cárcamo *et al.* 2000; Vos *et al.* 2011, 2013; De Smedt *et al.* 2018), we found that the presence of detritivores, indeed, significantly enhanced litter mass loss (**Chapter 4**). Importantly, presence of herb litter enhanced even more the mass loss of tree litter compared to detritivore addition alone (interaction effect, $p < 0.001$), and woodlice weight gain was significant under herb litter addition ($p = 0.02$), which might imply that the addition of herb litter could make tree litter more palatable to detritivores improving litter consumption (Blair & Stowasser 2009; Gerlach *et al.* 2014; Reis *et al.* 2018). On the contrary, our study seems to provide a different result, we found an additive effect of monospecific herb litter on mass loss of tree litter, while, the mass loss of the tree litter did not further increase when both herb litter and detritivores were added on the tree litter. Based on this, the increased mass loss was likely attributed to the mixture effects from the herb litter addition, but not through detritivore consumption. Weight gain of woodlice in the herb-tree litter mixtures could therefore probably be attributed to the feeding of detritivores on herb litter instead of tree litter because of their feeding preference for nutrient-rich litter (Slade & Riutta 2012).

*A. nemorosa* and *A. podagaria* are two common understorey forest species during spring and summer, respectively, both species are characterised as a nutrient-rich food resource for litter decomposers. Addition of *A. nemorosa* and *A. podagaria* herb litter to tree litter, consequently, directly and indirectly attracted a large amount of soil fauna, which led to a higher abundance and richness of soil fauna during the field experiment (**Chapter 5**). Nonetheless, the advantages in abundance and diversity did not result in higher decomposition efficiency, similar as we observed in our pervious study (**Chapter 4**), the higher survival rate of soil fauna (detritivores and omnivores) communities did not contribute to the decomposition of tree litter as it expected. As a nutrient-rich resource laying on top of more unpalatable tree litter, herbaceous litters should be the preferred choice for decomposers to feed on. Especially during periods when high quality leaf litter (fallen in autumn of the previous year) is mostly decomposed and nutrients are leached into soil. Therefore, the fresh inflow of spring and summer herbaceous litter could be an important food source for decomposers in spring and summer. In general, we can argue that herb litter addition would indirectly stimulate decomposition of tree litter via the decomposer communities, but our experiments showed that probably nutrient transfer from herb to tree litter is the main contributing mechanism to tree litter decomposition.

### 6.2.3 Litter mixture effects of herb litter on nutrient transfer

Next to the improved microenvironmental condition theory and interaction across trophic levels theory (Hättenschwiler *et al.* 2005) the nutrient transfer theory is a third plausible explanation for the increase in tree litter decomposition via herb litter addition. Corresponding to the synergistic and antagonistic additive effects of *A. nemorosa* and *A. podagaria* litter, respectively, on decomposition of recalcitrant tree litter we argue that this third theory is likely to be the most important. In the nutrient transfer theory, litter species with distinct chemical compositions allow nutrients to transfer from nutrient-rich litter towards nutrient-poor litter types, consequently stimulating the decomposition of co-occurring litter species within litter mixtures (Ganjegunte *et al.* 2005; Schimel & Hättenschwiler 2007; Bonanomi *et al.* 2014; Handa *et al.* 2014; Liu *et al.* 2020). Consistent with this theory, we found a significant antagonistic and synergistic effect when herb litter from respectively *A. nemorosa* and *A. podagaria* was added above tree litter. Nutrients and litter fragments can easily be transferred from herb litter on top to tree litter below via hydrological processes (**Chapter 4 and 5**). On the other hand, inhibitory effects were observed when the herb litter was added below the tree litter, as transfer from nutrient-rich to nutrient-poor litter species becomes less obvious (**Chapter 4**). The most likely explanation for these inhibitory effects is that the higher moisture content in the overlaying tree litter, caused by the underlaying herb litter, restricted oxygen permeability, in turn, inhibit the activity of microbial decomposers in the microcosms.

Nutrients in litter mixtures can be transferred passively via forest hydrological processes, such as leaching, or transferred actively through fungal mycelia networking (Gessner *et al.* 2010; Lummer *et al.* 2012; Bonanomi *et al.* 2014). In this thesis, we focused on the passive nutrients transfer of *A. nemorosa* and *A. podagaria* litter to underlying tree litter. The effects of herb addition were stronger when mixtures with contrasting nutrient content (slowly-decomposing tree litter) was considered. On the one hand, leaching fragments and substances are more easily intercepted by slowly-decomposing litter compared to rapidly-decomposing litter (Santos *et al.* 2018). The remaining tissues in high quality litter (e.g. *T.*

*platyphyllos* and *F. excelsior*) were mostly leaf nerves after about 250 days of incubation, thus the nutrient and fragment interception capacity are reduced. On the other hand, the recalcitrant litter with herb litter addition, not only provided various food resource, but also larger habitat for decomposers. The *A. nemorosa* and *A. podagaria* represent two distinct labile litter types: N-rich and P-K-rich, respectively. Therefore, the mixture effects driven by the nutrient transfer theory should relate to the characteristics of the nutrient content in the herb litter. Addition of N-rich herb litter (i.e. *A. nemorosa* litter) resulted in an antagonistic effect on the decomposition of *F. sylvatica*, *Q. robur*, and *A. pseudoplatanus* litter (Fig. 5.3), this was in accordance with nitrogen enrichment effects. The higher N input from N-rich herb litter might not alter the N-status in the decomposing environment of tree litter, as N-limitation is not likely to occur in a region with high N-deposition such as northern Belgium (Bobbink *et al.* 1998; Ganjegunte *et al.* 2005; Knorr *et al.* 2005). On the contrary, P- and K-enrichment via *A. podagaria* litter is likely to enhance decomposition of low quality litter (Feng *et al.* 2018; Ochoa-Hueso *et al.* 2019) as shown is our field experiment (**Chapter 5**).

In contrast to the selective transfer of nutrients by microbes (for example via fungi growing in nutrient-poor litter taking up limiting nutrients by extending their hyphae to alternative nutrient-rich litter, achieving targeted nutrient transfer), the passive transfer via leaching and subsequent transport can also involve inhibitory compounds such as phenolics and tannins, which may negatively influence decomposition on other litter types (Gartner & Cardon 2004; Liu *et al.* 2020). the water-holding capacity of *A. nemorosa* litter might lower compared to the *A. podagaria* litter, which might also explain the reverse effects that these two herbaceous litter led (Pérez-Suárez et al., 2012). On the other hand, compared to *A. podagaria* litter, the *A. nemorosa* litter contains a higher number of deterrent compounds (e.g., tannins, which are considered unpalatable for decomposers). Lukianchuk et al. (2017) reported that *A. nemorosa* contains glycosides, a type of tannins, which are generally considered toxic to some detritivores such as Gastropoda (Mølgaard 1986). On the contrary, *A. podagaria* litter can make the tree litter, especially recalcitrant litter, more palatable for decomposers. For example, we observed a comparatively higher number of Diptera larva (which area important decomposers) in summer herb litter and the underlying tree litter.

## 6.3 Implications for forest management

The understorey vegetation has been considered an adverse factor in silvicultural systems  because of its inhibitory effects on tree seedling establishment, and as a fire risk factor in fire-prone environments (Hull *et al.* 2008; Mitchell *et al.* 2009; Vild *et al.* 2018).  Additionally, some of the herb materials could be used for fuel, livestock feeding, medical using, for tea making (e.g., in Russia, leaves of *Chamaenerion angustifolium* is used to make fermented Ivan Chai tea (Kolosova *et al.* 2020)). These understorey vegetation management practices have been applied for millennia. However, from the 19th century on, these forest management practices were increasingly reported as unsustainable for forest ecosystems, for example, due to a decline in soil organic matter and nutrient content, and strongly limiting the abundance and diversity of decomposers on the forest floor (Bokhorst *et al.* 2014; Jang *et al.* 2015).

The understorey layer in forests generally maintains a metastable soil microclimate, by buffering the macroclimate in a similar was as the overstorey, to create a forest interior microclimate (Zellweger *et al.* 2019; Vanneste *et al.* 2020). The regulatory function of the understorey layer on the soil thermal and moisture status has been recently studied in subtropical and boreal forest (Matsushima & Chang 2007; Wu *et al.* 2011; Zhao *et al.* 2013; Wang *et al.* 2014), but it remains understudied in temperate forests and cannot be extrapolated from other biomes since the understorey and overstorey compositions in temperate forests are obviously very different. Temperate forest canopies are mostly covered by broadleaved deciduous tree species, and the understorey layer is often dominated by ephemeral herbaceous species (with short growing seasons). To fill this knowledge gap, in Chapter 2 and 3, we investigated the regulatory effects of understorey vegetation on the soil microclimate, and its further effects on the decomposition.

Our findings suggest that, under the current climate conditions, understorey removal would not affect litter decomposition via the short-term regulation of the soil microclimate, but we found that the C sequestration and nutrient releasing would be slower under a denser herbaceous vegetation cover. In the context of a warming climate and management practices that focus on more open canopies (increase in light availability), the composition of the understorey layer might modify the soil C emissions. We found that the positive reaction of understorey communities (via increased vegetation cover) to warming and increased light availability, in turn, led to a negative effect on litter decomposition. Therefore, the functional role of understorey communities on the soil microclimate and degradation of soil organic matter cannot be ignored in forest management practices.

Previous studies rarely take the understorey herb layer into account and the heterogeneity of the understorey layer is also rarely mentioned when studying the carbon and nutrient recycling in temperate forests. Here I show the potential effects of litter mixtures (tree plus herb litter) on the decomposition environment and its role in nutrient transfer. To illustrate this, being the most abundant understorey species in the mesotrophic forest types of the Aelmoeseneie forest, the above-ground biological production of *A. nemorosa* is about 24.5 kg N ha$^{-1}$, which should be considered as an additional and substantial N source on the forest litter layer. This amount is even higher than the annual atmospheric N deposition in this region (about 20 kg N ha$^{-1}$). Importantly, the N being locked in *A. nemrosa* biomass is released in a short period of time, because, as a labile litter, the *A. nemorosa* litter will be rapidly decomposed. However, removal of an N-rich herb, such as *A. nemorosa*, would decrease C sequestration in temperate forest soils, while removal of an P-K-rich herb such as *A. podagraria,* would have opposite effect on C sequestration. Thus, the forest managers should consider these herbaceous litter as an important nutrient source for the trees in the stand.

## 6.4 Suggestions for further research

As an important functional component in forest ecosystems, the understorey layer plays an essential role in C and nutrient cycling, either directly by reducing the circulating period (fast growth and rapid decomposition), or indirectly by regulating soil microclimate and providing litter mixture effects. The field experiments of this thesis were executed in an ancient temperate mixed deciduous forest, with seasonal understorey dominated by *A. nemorosa* and *A. podagraria* for a two-year period. However, it would be interesting to know how these ephemeral understorey species influence soil physical and chemical properties and soil nutrients transfer in the longer term. We can assume that nutrient content in the topsoil will gradually decrease after understorey layer removed continuously, as the understorey removal could lead to a higher surface runoff and nutrients leaching to deeper soil layer. We encourage to conduct our experiments in other forest biomes differing in overstory and understorey species, diversity, or forest structure.

### *The effect of the understorey layer on the soil microbial community*

We found that environmental factors (e.g., warming, light) are strengthened by understorey vegetation which could strongly influence microbial activity in soils, further resulting in altered turnover rates of forest litter. However, the directions and net effects are unknown. Therefore, we encourage future research to focus on the reaction of the microbial community to the changes in the understorey layer (e.g. by applying PLFA, sequencing techniques or eDNA metabarcoding).

*Thermal-driven migration of soil fauna*

Not only the microbial communities can be affected by the understorey vegetation, but also the macrofauna. During the teabag incubation, we collected more woodlice in the understorey removal plots than in the understorey presence plot, indicating a thermal-driven migration from understorey cover to understorey gap, there might be a returning activity with temperature increasing and with herbs senescing. This could indicate the importance of forest understorey for soil fauna behavior which is currently unstudied but has potential large consequences for litter decomposition between structurally very different forest types or e.g. in forest edges compared to forest interiors.

*Diversity and identity effect of understorey species on soil C sequestration and nutrient cycling*

Concerning mixture effects, different herbaceous species may have different additive effects on nutrient cycling, due to their species-specific nutrient content. It would be interesting to trace nutrient flows between leaves with a different nutrient status (e.g. by using isotopic tracing), and how this nutrient transfer influences litter decomposition.

## 6.5 General conclusion

In summary, we studied the **regulatory effects** of understorey layer on tree litter decomposition via its microclimatic regulation, and through **mixture effects** of two dominant understorey herbaceous species (*A. nemorosa* and *A. podagaria*) on the decomposition of five common overstorey tree species (*F. sylvatica*, *Q. robur*, *A. pseudoplatanus*, *F. excelsior*, and *Tilia platyphyllos*) in a temperate forest in northern Belgium. We observed that the understorey layer influences near-surface microclimate, by regulating soil temperature and humidity at forest floor level (**Chapter 2**) and through the seasonality of litter input (**Chapter 4**). Moreover, the microclimate regulation of the understorey layer performed in accordance to the understorey cover, for instance the higher cover generally created a colder and drier soil microenvironment, which is known to have negative influence on degradation of organic matter (Prescott 2010; Butenschoen *et al.* 2011). We observed a consistent negative correlation between understorey cover and litter decomposition under field conditions (**Chapter 2** and **3**). Presence of herb litter on tree litter had a significant effect on litter moisture, soil fauna community composition and tree litter nutrient content during decomposition. However, changes in microenvironmental conditions in the litter layer and the increase in soil fauna abundance did not accelerate the decomposition rates of tree litter as expected, instead, nutrient transfer from herb litter towards tree litter played a major role for tree litter decomposition. Specifically, the N-rich herb (*A. nemorosa*) showed antagonistic effects on the decomposition of tree litter, whereas the P-K-rich herb (*A. podagaria*) showed synergistic effects on decomposition of tree litter, particularly for recalcitrant litter species, such as *F. sylvatica* and *Q. robur* (**Chapter 4** and **5**). These findings contribute to the evaluation of the functional role of understorey vegetation on nutrient cycling, in temperate forests.

# 7

# References

A'Bear, A. D., Boddy, L., & Jones, T. H. (2013). Bottom-up determination of soil collembola diversity and population dynamics in response to interactive climatic factors. *Oecologia*, *173*(3), 1083-1087. doi: 10.1007/s00442-013-2662-3

Aerts, R. (1997). Climate , Leaf Litter Chemistry and Leaf Litter Decomposition in Terrestrial Ecosystems : A Triangular Relationship. *Oikos*, *79*(3), 439-449. doi: 10.2307/3546886

Almagro, M., Maestre, F. T., Martínez-López, J., Valencia, E., & Rey, A. (2015). Climate change may reduce litter decomposition while enhancing the contribution of photodegradation in dry perennial Mediterranean grasslands. *Soil Biology and Biochemistry*, *90*, 214-223. doi: 10.1016/j.soilbio.2015.08.006

Angst, G., Mueller, K. E., Kögel-Knabner, I., Freeman, K. H., & Mueller, C. W. (2017). Aggregation controls the stability of lignin and lipids in clay-sized particulate and mineral associated organic matter. *Biogeochemistry*, *132*(3), 307-324. doi: 10.1007/s10533-017-0304-2

Attiwill, P. M. (1968). The Loss of Elements from Decomposing Litter. *Ecology*, *49*(1), 142-145. doi: 10.2307/1933568

Aubert, M., Margerie, P., Trap, J., & Bureau, F. (2010). Aboveground-belowground relationships in temperate forests: Plant litter composes and microbiota orchestrates. *Forest Ecology and Management*, *259*(3), 563-572. doi: 10.1016/j.foreco.2009.11.014

Austin, A. T., Araujo, P. I., & Leva, P. E. (2009). Interaction of position, litter type, and water pulses on decomposition of grasses from the semiarid Patagonian steppe. *Ecology*, *90*(9), 2642-2647. doi: 10.1890/08-1804.1

Austin, A. T., & Vivanco, L. (2006). Plant litter decomposition in a semi-arid ecosystem controlled by photodegradation. *Nature*, *442*(7102), 555-558. doi: 10.1038/nature05038

Badri, D. V., & Vivanco, J. M. (2009). Regulation and function of root exudates. *Plant, Cell and Environment*, *32*(6), 666-681. doi: 10.1111/j.1365-3040.2009.01926.x

Baeten, L., Verstraeten, G., De Frenne, P., Vanhellemont, M., Wuyts, K., Hermy, M., & Verheyen, K. (2010). Former land use affects the nitrogen and phosphorus concentrations and biomass of forest herbs. *Plant Ecology*, *212*(5), 901-909. doi: 10.1007/s11258-010-9876-9

Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S., & Vivanco, J. M. (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology*, *57*, 233-266. doi: 10.1146/annurev.arplant.57.032905.105159

Barbier, S., Gosselin, F., & Balandier, P. (2008). Influence of tree species on understory vegetation diversity and mechanisms involved-A critical review for temperate and boreal forests. *Forest Ecology and Management*, *254*(1), 1-15. doi: 10.1016/j.foreco.2007.09.038

Bastow, J. L., Preisser, E. L., & Strong, D. R. (2008). Holcus lanatus invasion slows decomposition through its interaction with a macroinvertebrate detritivore, Porcellio scaber. *Biological Invasions*, *10*(2), 191-199. doi: 10.1007/s10530-007-9122-0

Bates, D., Mächler, M., Bolker, B. M., & Walker, S. C. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*, 1-48. doi: 10.18637/jss.v067.i01

Berg, B., Davey, M. P., de Marco, A., Emmett, B., Faituri, M., Hobbie, S. E., … Virzo De Santo, A. (2010). Factors influencing limit values for pine needle litter decomposition: A synthesis for boreal and temperate pine forest systems. *Biogeochemistry*, *100*(1), 57-73. doi: 10.1007/s10533-009-9404-y

Berg, Björn. (2000). Litter decomposition and organic matter turnover in northern forest soils. *Forest Ecology and Management*, *1333*(1), 13-22. doi: 10.2134/jeq1993.00472425002200040003x

Berg, Björn. (2014). Decomposition patterns for foliar litter - A theory for influencing factors. *Soil Biology and Biochemistry*, *78*, 222-232. doi: 10.1016/j.soilbio.2014.08.005

Berger, T. W., & Berger, P. (2014). Does mixing of beech (Fagus sylvatica) and spruce (Picea abies) litter hasten decomposition? *Plant and Soil*, *377*(1-2), 217-234. doi: 10.1007/s11104-013-2001-9

Blair, B. C., & Stowasser, A. (2009). Impact of Lonicera maackii on decomposition rates of native leaf litter in a southwestern Ohio woodland. *Ohio Journal of Science*, *109*(3), 43-47.

Blondeel, H., Perring, M. P., Bergès, L., Brunet, J., Decocq, G., Depauw, L., … Verheyen, K. (2019). Context-Dependency of Agricultural Legacies in Temperate Forest Soils. *Ecosystems*, *22*(4), 781-795. doi: 10.1007/s10021-018-0302-9

Blondeel, H., Perring, M. P., Depauw, L., De Lombaerde, E., Landuyt, D., De Frenne, P., & Verheyen, K. (2020). Light and warming drive forest understorey community development in different environments. *Global Change Biology*, *26*(3), 1681-1696. doi: 10.1111/gcb.14955

Blondeel, H., Remy, E., Perring, M. P., Landuyt, D., Bodé, S., De Frenne, P., … Verheyen, K. (2019). Larger direct than indirect effects of multiple environmental changes on leaf nitrogen of forest herbs. *Plant and Soil*, *445*(1-2), 199-216. doi: 10.1007/s11104-019-04298-1

Bobbink, R., Hornung, M., & Roelofs, J. G. M. (1998). The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. *Journal of Ecology*, *86*(8), 717-738. doi: 10.1890/0012-9658(1999)080[2170:PMCONC]2.0.CO;2

Boča, A., Van Miegroet, H., & Gruselle, M.-C. (2014). Forest Overstory Effect on Soil Organic Carbon Storage: A Meta-analysis. *Soil Science Society of America Journal*, *78*(S1), S35-S47. doi: 10.2136/sssaj2013.08.0332nafsc

Bodenhofer, U., Kothmeier, A., & Hochreiter, S. (2011). Apcluster: An R package for affinity propagation clustering. *Bioinformatics, 27*(17), 2463-2464. doi: 10.1093/bioinformatics/btr406

Bokhorst, S., Wardle, D. A., Nilsson, M. C., & Gundale, M. J. (2014). Impact of understory mosses and dwarf shrubs on soil micro-arthropods in a boreal forest chronosequence. *Plant and Soil, 379*(1-2), 121-133. doi: 10.1007/s11104-014-2055-3

Bonan, G. B. (2008). Forests and Climate Change: Forcings, Feedbacks, and the Climate Benefits of Forests. *Science, 320*(5882), 1444-1449. doi: 10.1126/science.1155121

Bonanomi, G., Capodilupo, M., Incerti, G., & Mazzoleni, S. (2014). Nitrogen transfer in litter mixture enhances decomposition rate, temperature sensitivity, and C quality changes. *Plant and Soil, 381*(1-2), 307-321. doi: 10.1007/s11104-014-2119-4

Bonner, M. T., Castro, D., Schneider, A. N., Sundström, G., Hurry, V., Street, N. R., & Näsholm, T. (2019). Why does nitrogen addition to forest soils inhibit decomposition? *Soil Biology and Biochemistry, 137,* 107570. doi: 10.1016/j.soilbio.2019.107570

Bray, J. R., & Gorham, E. (1964). Litter Production in Forests of the World. *Advances in Ecological Research*, *2*(C), 101-157. doi: 10.1016/S0065-2504(08)60331-1

Butenschoen, O., Scheu, S., & Eisenhauer, N. (2011). Interactive effects of warming, soil humidity and plant diversity on litter decomposition and microbial activity. *Soil Biology and Biochemistry*, *43*(9), 1902-1907. doi: 10.1016/j.soilbio.2011.05.011

Callaway, R. M., & Ridenour, W. M. (2004). Novel weapons: Invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment*, *2*(8), 436-443. doi: 10.1890/1540-9295(2004)002[0436:NWISAT]2.0.CO;2

Cárcamo, H. A., Abe, T. A., Prescott, C. E., Holl, F. B., & Chanway, C. P. (2000). Influence of millipedes on litter decomposition, N mineralization, and microbial communities in a coastal forest in British Columbia, Canada. *Canadian Journal of Forest Research*, *30*(5), 817-826. doi: 10.1139/x00-014

Carrillo, Y., Ball, B. A., Bradford, M. A., Jordan, C. F., & Molina, M. (2011). Soil fauna alter the effects of litter composition on nitrogen cycling in a mineral soil. *Soil Biology and Biochemistry*, *43*(7), 1440-1449. doi: 10.1016/j.soilbio.2011.03.011

Cesarz, S., Craven, D., Dietrich, C., & Eisenhauer, N. (2016). Effects of soil and leaf litter quality on the biomass of two endogeic earthworm species. *European Journal of Soil Biology*, *77*, 9-16. doi: 10.1016/j.ejsobi.2016.09.002

Cesarz, S., Ruess, L., Jacob, M., Jacob, A., Schaefer, M., & Scheu, S. (2013). Tree species diversity versus tree species identity: Driving forces in structuring forest food webs as indicated by soil nematodes. *Soil Biology and Biochemistry, 62,* 36-45. doi: 10.1016/j.soilbio.2013.02.020

Chapman, S. K., Newman, G. S., Hart, S. C., Schweitzer, J. A., & Koch, G. W. (2013). Leaf Litter Mixtures Alter Microbial Community Development: Mechanisms for Non-Additive Effects in Litter Decomposition. *PLoS ONE, 8*(4), e62671. doi: 10.1371/journal.pone.0062671

Chen, H. Y. H., Biswas, S. R., Sobey, T. M., Brassard, B. W., & Bartels, S. F. (2018). Reclamation strategies for mined forest soils and overstorey drive understorey vegetation. *Journal of Applied Ecology, 55*(2), 926-936. doi: 10.1111/1365-2664.13018

Chomel, M., Guittonny-Larchevêque, M., DesRochers, A., & Baldy, V. (2016). Effect of mixing herbaceous litter with tree litters on decomposition and N release in boreal plantations. *Plant and Soil*, *398*(1-2), 229-241. doi: 10.1007/s11104-015-2648-5

Christiansen, C. T., Haugwitz, M. S., Priemé, A., Nielsen, C. S., Elberling, B., Michelsen, A., … Blok, D. (2017). Enhanced summer warming reduces fungal decomposer diversity and litter mass loss more strongly in dry than in wet tundra. *Global Change Biology*, *23*(1), 406-420. doi: 10.1111/gcb.13362

Cleveland, C. C., Reed, S. C., Keller, A. B., Nemergut, D. R., O'Neill, S. P., Ostertag, R., & Vitousek, P. M. (2014). Litter quality versus soil microbial community controls over decomposition: A quantitative analysis. *Oecologia*, *174*(1), 283-294. doi: 10.1007/s00442-013-2758-9

Clinton, B. D. (2003). Light, temperature, and soil moisture responses to elevation, evergreen understory, and small canopy gaps in the southern Appalachians. *Forest Ecology and Management*, *186*(1-3), 243-255. doi: 10.1016/S0378-1127(03)00277-9

Coates, K. D., Emmingham, W. H., & Radosevich, S. R. (1991). Conifer-seedling success and microclimate at different levels of herb and shrub cover in a Rhododendron - Vaccinium - Menziesia community of south central British Columbia. *Canadian Journal of Forest Research, 21*(6), 858-866. doi: 10.1139/x91-120

Cornelissen, J. H.C., Pérez-Harguindeguy, N., Gwynn-Jones, D., Díaz, S., Callaghan, T. V., & Aerts, R. (2000). Autumn leaf colours as indicators of decomposition rate in sycamore (Acer pseudoplatanus L.). *Plant and Soil*, *225*(1-2), 33-38. doi: 10.1023/A:1026530214258

Cornelissen, J. H.C., Quested, H. M., Gwynn-Jones, D., Van Logtestijn, R. S. P., De Beus, M. A. H., Kondratchuk, A., … Aerts, R. (2004). Leaf digestibility and litter decomposability are related in a wide range of subarctic plant species and types. *Functional Ecology*, *18*(6), 779-786. doi: 10.1111/j.0269-8463.2004.00900.x

Cornelissen, J. H.C., & Thompson, K. (1997). Functional leaf attributes predict litter decomposition rate in herbaceous plants. *New Phytologist*, *135*(1), 109-114. doi: 10.1046/j.1469-8137.1997.00628.x

Cornelissen, Johannes H.C., Bodegom, P. M. Van, Aerts, R., Callaghan, T. V., Logtestijn, R. S. P. Van, Alatalo, J., … Team, M. O. L. (2007). Global negative vegetation feedback to climate warming responses of leaf litter decomposition rates in cold biomes. *Ecology Letters*, *10*(7), 619-627. doi: 10.1111/j.1461-0248.2007.01051.x

Cornwell, W. K., Cornelissen, J. H. C., Amatangelo, K., Dorrepaal, E., Eviner, V. T., Godoy, O., … Westoby, M. (2008). Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecology Letters, 11*(10), 1065-1071. doi: 10.1111/j.1461-0248.2008.01219.x

Coulis, M., Hättenschwiler, S., Fromin, N., & David, J. F. (2013). Macroarthropod-microorganism interactions during the decomposition of Mediterranean shrub litter at different moisture levels. *Soil Biology and Biochemistry*, *64*, 114-121. doi: 10.1016/j.soilbio.2013.04.012

Coûteaux, M. M., Bottner, P., & Berg, B. (1995). Litter decomposition, climate and liter quality. *Trends in Ecology & Evolution*, *10*(2), 63-66. doi: 10.1016/S0169-5347(00)88978-8

Dang, C. K., Schindler, M., Chauvet, E., & Gessner, M. O. (2009). Temperature oscillation coupled with fungal community shifts can modulate warming effects on litter decomposition. *Ecology*, *90*(1), 122-131. doi: 10.1890/07-1974.1

David, J. F. (2014). The role of litter-feeding macroarthropods in decomposition processes: A reappraisal of common views. *Soil Biology and Biochemistry*, *76*, 109-118. doi: 10.1016/j.soilbio.2014.05.009

David, J. F., & Gillon, D. (2002). Annual feeding rate of the millipede Glomeris marginata on holm oak (Quercus ilex) leaf litter under Mediterranean conditions. *Pedobiologia*, *46*(1), 42-52. doi: 10.1078/0031-4056-00112

David, J. F., & Gillon, D. (2009). Combined effects of elevated temperatures and reduced leaf litter quality on the life-history parameters of a saprophagous macroarthropod. *Global Change Biology*, *15*(1), 156-165. doi: 10.1111/j.1365-2486.2008.01711.x

David, J. F., & Handa, I. T. (2010). The ecology of saprophagous macroarthropods (millipedes, woodlice) in the context of global change. *Biological Reviews, 85*(4), 881-895. doi: 10.1111/j.1469-185X.2010.00138.x

Davidson, E. A., & Janssens, I. A. (2006). Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature, 440*(7081), 165-173. doi: 10.1038/nature04514

Dayton, P. K. (1975). Experimental Evaluation of Ecological Dominance in a Rocky Intertidal Algal Community. *Ecological Monographs*, *45*(2), 137-159. doi: 10.2307/1942404

De Deyn, G. B., Raaijmakers, C. E., Van Ruijven, J., Berendse, F., & Van Der Putten, W. H. (2004). Plant species identity and diversity effects on different trophic levels of nematodes in the soil food web. *Oikos*, *106*(3), 576-586. doi: 10.1111/j.0030-1299.2004.13265.x

De Forest, J. L., Zak, D. R., Pregitzer, K. S., & Burton, A. J. (2014). Atmospheric Nitrate Deposition, Microbial Community Composition, and Enzyme Activity in Northern Hardwood Forests. *Soil Science Society of America Journal*, *68*(1), 132-138. doi: 10.2136/sssaj2004.1320

De Frenne, P., Graae, B. J., Kolb, A., Brunet, J., Chabrerie, O., Cousins, S. A. O., … Verheyen, K. (2010). Significant effects of temperature on the reproductive output of the forest herb Anemone nemorosa L. *Forest Ecology and Management*, *259*(4), 809-817. doi: 10.1016/j.foreco.2009.04.038

De Frenne, Pieter, De Schrijver, A., Bente J. Graae, Gruwez, R., Tack, W., Vandelook, F., … Kris, V. (2010). The use of open-top chambers in forests for evaluating warming effects on herbaceous understorey plants. *Ecological Research*, *25*(1), 163-171. doi: 10.1007/s11284-009-0640-3

De Frenne, Pieter, Rodríguez-Sánchez, F., De Schrijver, A., Coomes, D. A., Hermy, M., Vangansbeke, P., & Verheyen, K. (2015). Light accelerates plant responses to warming. *Nature Plants, 1*(9), 15110. doi: 10.1038/nplants.2015.110

De Frenne, Pieter, Zellweger, F., Rodríguez-Sánchez, F., Scheffers, B. R., Hylander, K., Luoto, M., … Lenoir, J. (2019). Global buffering of temperatures under forest canopies. *Nature Ecology and Evolution, 3*(5), 744-749. doi: 10.1038/s41559-019-0842-1

De Lombaerde, E., Blondeel, H., Baeten, L., Landuyt, D., Perring, M. P., Depauw, L., … Verheyen, K. (2020). Light, temperature and understorey cover predominantly affect early life stages of tree seedlings in a multifactorial mesocosm experiment. *Forest Ecology and Management*, *461*, 117907. doi: 10.1016/j.foreco.2020.117907

De Long, J. R., Dorrepaal, E., Kardol, P., Nilsson, M. C., Teuber, L. M., & Wardle, D. A. (2016). Understory plant functional groups and litter species identity are stronger drivers of litter decomposition than warming along a boreal forest post-fire successional gradient. *Soil Biology and Biochemistry, 98,* 159-170. doi: 10.1016/j.soilbio.2016.04.009

De Smedt, P., Wasof, S., Van de Weghe, T., Hermy, M., Bonte, D., & Verheyen, K. (2018). Macro-detritivore identity and biomass along with moisture availability control forest leaf litter breakdown in a field experiment. *Applied Soil Ecology, 131,* 47-54. doi: 10.1016/j.apsoil.2018.07.010

Del Tredici, P. (2001). Sprouting in temperate trees: A morphological and ecological review. *Botanical Review*, *67*(2), 121-140. doi: 10.1007/BF02858075

Delgado-Baquerizo, M., García-Palacios, P., Milla, R., Gallardo, A., & Maestre, F. T. (2015). Soil characteristics determine soil carbon and nitrogen availability during leaf litter decomposition regardless of litter quality. *Soil Biology and Biochemistry, 81,* 134-142. doi: 10.1016/j.soilbio.2014.11.009

Deutsch, E. S., Bork, E. W., & Willms, W. D. (2010). Separation of grassland litter and ecosite influences on seasonal soil moisture and plant growth dynamics. *Plant Ecology*, *209*(1), 135-145. doi: 10.1007/s11258-010-9729-6

Didion, M., Repo, A., Liski, J., Forsius, M., Bierbaumer, M., & Djukic, I. (2016). Towards harmonizing leaf litter decomposition studies using standard tea bags-a field study and model application. *Forests*, *7*(8), 1-12. doi: 10.3390/f7080167

Dilly, O., Bartsch, S., Rosenbrock, P., Buscot, F., & Munch, J. C. (2001). Shifts in physiological capabilities of the microbiota during the decomposition of leaf litter in a black alder (Alnus glutinosa (Gaertn.) L.) forest. *Soil Biology and Biochemistry*, *33*(7-8), 921-930. doi: 10.1016/S0038-0717(00)00239-X

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

Easlon, H. M., & Bloom, A. J. (2014). Easy Leaf Area: Automated Digital Image Analysis for Rapid and Accurate Measurement of Leaf Area. *Applications in Plant Sciences*, *2*(7), 1400033. doi: 10.3732/apps.1400033

Eisenhauer, N., Beßler, H., Engels, C., Gleixner, G., Habekost, M., Milcu, A., … Scheu, S. (2010). Plant diversity effects on soil microorganisms support the singular hypothesis. *Ecology*, *91*(2), 485-496. doi: 10.1890/08-2338.1

Ellert, B. H., Dyck, M. F., Janzen, H., May, W. E., Beyaert, R. P., Helgason, B. L., … Gregorich, E. G. (2016). Litter decay controlled by temperature, not soil properties, affecting future soil carbon. *Global Change Biology*, *23*(4), 1725-1734. doi: 10.1111/gcb.13502

Erktan, A., Balmot, J., Merino-Martín, L., Monnier, Y., Pailler, F., Coq, S., … Le Bissonnais, Y. (2017). Immediate and long-term effect of tannins on the stabilization of soil aggregates. *Soil Biology and Biochemistry*, *105*, 197-205. doi: 10.1016/j.soilbio.2016.11.017

Fanin, N., Bezaud, S., Sarneel, J. M., Cecchini, S., Nicolas, M., & Augusto, L. (2020). Relative Importance of Climate, Soil and Plant Functional Traits During the Early Decomposition Stage of Standardized Litter. *Ecosystems*, *23*(5), 1004-1018. doi: 10.1007/s10021-019-00452-z

Fanin, N., Kardol, P., Farrell, M., Kempel, A., Ciobanu, M., Nilsson, M., … Wardle, D. A. (2019). Effects of plant functional group removal on structure and function of soil communities across contrasting ecosystems. *Ecology Letters*, *22*(7), 1095-1103. doi: 10.1111/ele.13266

Feller, I. C., McKee, K. L., Whigham, D. F., & O’Neill, J. P. (2003). Nitrogen vs. phosphorus limitation across an ecotonal gradient in a mangrove forest. *Biogeochemistry*, *62*(2), 145-175. doi: 10.1023/A:1021166010892

Feng, H., Xue, L., & Chen, H. (2018). Responses of decomposition of green leaves and leaf litter to stand density, N and P additions in Acacia auriculaeformis stands. *European Journal of Forest Research*, *137*(6), 819-830. doi: 10.1007/s10342-018-1142-z

Fernandez, C. W., Langley, J. A., Chapman, S., McCormack, M. L., & Koide, R. T. (2016). The decomposition of ectomycorrhizal fungal necromass. *Soil Biology and Biochemistry*, *93*, 38-49. doi: 10.1016/j.soilbio.2015.10.017

Fierer, N., Craine, J. M., McLauchlan, K., & Schimel, J. P. (2005). Litter quality and the temperature sensitivity of decomposition. *Ecology*, *86*(2), 320-326. doi: 10.1890/04-1254

Fioretto, A., Di Nardo, C., Papa, S., & Fuggi, A. (2005). Lignin and cellulose degradation and nitrogen dynamics during decomposition of three leaf litter species in a Mediterranean ecosystem. *Soil Biology and Biochemistry*, *37*(6), 1083-1091. doi: 10.1016/j.soilbio.2004.11.007

Fontaine, S., Mariotti, A., & Abbadie, L. (2003). The priming effect of organic matter: A question of microbial competition? *Soil Biology and Biochemistry*, *35*(6), 837-843. doi: 10.1016/S0038-0717(03)00123-8

Fontana, S., Berg, M. P., & Moretti, M. (2019). Intraspecific niche partitioning in macrodetritivores enhances mixed leaf litter decomposition. *Functional Ecology*, *33*(12), 2391-2401. doi:

10.1111/1365-2435.13448

Foss, A. R., Mattson, W. J., & Trier, T. M. (2013). Effects of Elevated CO2 Leaf Diets on Gypsy Moth (Lepidoptera: Lymantriidae) Respiration Rates. *Environmental Entomology*, *42*(3), 503-514. doi: 10.1603/en12074

Frøseth, R. B., & Bleken, M. A. (2015). Effect of low temperature and soil type on the decomposition rate of soil organic carbon and clover leaves, and related priming effect. *Soil Biology and Biochemistry, 80*, 156-166. doi: 10.1016/j.soilbio.2014.10.004

Frouz, J. (2018). Effects of soil macro- and mesofauna on litter decomposition and soil organic matter stabilization. *Geoderma*, *332*, 161-172. doi: 10.1016/j.geoderma.2017.08.039

Fu, Q., Cheng, L., Guo, Y., & Turgeon, R. (2011). Phloem loading strategies and water relations in trees and herbaceous plants. *Plant Physiology*, *157*(3), 1518-1527. doi: 10.1104/pp.111.184820

Fu, X., Yang, F., Wang, J., Di, Y., Dai, X., Zhang, X., & Wang, H. (2015). Understory vegetation leads to changes in soil acidity and in microbial communities 27years after reforestation. *Science of the Total Environment*, *502*, 280-286. doi: 10.1016/j.scitotenv.2014.09.018

Gallo, M., Amonette, R., Lauber, C., Sinsabaugh, R. L., & Zak, D. R. (2004). Microbial community structure and oxidative enzyme activity in nitrogen-amended north temperate forest soils. *Microbial Ecology*, *48*(2), 218-229. doi: 10.1007/s00248-003-9001-x

Ganjegunte, G. K., Condron, L. M., Clinton, P. W., & Davis, M. R. (2005). Effects of mixing radiata pine needles and understory litters on decomposition and nutrients release. *Biology and Fertility of Soils*, *41*(5), 310-319. doi: 10.1007/s00374-005-0851-x

Gannes, L. Z., O'Brien, D. M., & Del Rio, C. M. (1997). Stable isotopes in animal ecology: Assumptions, caveats, and a call for more laboratory experiments. *Ecology*, *78*(4), 1271-1276. doi: 10.1890/0012-9658(1997)078[1271:SIIAEA]2.0.CO;2

García-Palacios, P., Maestre, F. T., Kattge, J., & Wall, D. H. (2013). Climate and litter quality differently modulate the effects of soil fauna on litter decomposition across biomes. *Ecology Letters*, *16*(8), 1045-1053. doi: 10.1111/ele.12137

García-Palacios, P., Mckie, B. G., Handa, I. T., Frainer, A., & Hättenschwiler, S. (2016). The importance of litter traits and decomposers for litter decomposition: A comparison of aquatic and terrestrial ecosystems within and across biomes. *Functional Ecology*, *30*(5), 819-829. doi: 10.1111/1365-2435.12589

García-Palacios, P., Shaw, E. A., Wall, D. H., & Hättenschwiler, S. (2016). Temporal dynamics of biotic and abiotic drivers of litter decomposition. *Ecology Letters, 19*(5), 554-563. doi: 10.1111/ele.12590

Gartner, T. B., & Cardon, Z. G. (2004). Decomposition dynamics in mixed-species leaf litter. *Oikos, 104*(2), 230-246. doi: 10.1111/j.0030-1299.2004.12738.x

Gerlach, A., Russell, D. J., Jaeschke, B., & Römbke, J. (2014). Feeding preferences of native terrestrial isopod species (Oniscoidea, Isopoda) for native and introduced leaf litter. *Applied Soil Ecology, 83*, 95-100. doi: 10.1016/j.apsoil.2014.02.006

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

Gilliam, F. S. (2007). The Ecological Significance of the Herbaceous Layer in Temperate Forest Ecosystems. *BioScience, 57*(10), 845-858. doi: 10.1641/b571007

Gilliam, F. S., Billmyer, J. H., Walter, C. A., & Peterjohn, W. T. (2016). Effects of excess nitrogen on biogeochemistry of a temperate hardwood forest: Evidence of nutrient redistribution by a forest understory species. *Atmospheric Environment, 146*, 261-270. doi: 10.1016/j.atmosenv.2016.04.007

Giuggiola, A., Zweifel, R., Feichtinger, L. M., Vollenweider, P., Bugmann, H., Haeni, M., & Rigling, A. (2018). Competition for water in a xeric forest ecosystem - Effects of understory removal on soil micro-climate, growth and physiology of dominant Scots pine trees. *Forest Ecology and Management, 409*, 241-249. doi: 10.1016/j.foreco.2017.11.002

Glassman, S. I., Weihe, C., Li, J., Albright, M. B. N., Looby, C. I., Martiny, A. C., … Martiny, J. B. H. (2018). Decomposition responses to climate depend on microbial community composition. *Proceedings of the National Academy of Sciences of the United States of America, 115*(47), 11994-11999. doi: 10.1073/pnas.1811269115

González, G., Lodge, D. J., Richardson, B. A., & Richardson, M. J. (2014). A canopy trimming experiment in Puerto Rico: The response of litter decomposition and nutrient release to canopy opening and debris deposition in a subtropical wet forest. *Forest Ecology and Management, 332*, 32-46. doi: 10.1016/j.foreco.2014.06.024

Gosz, J. R., Likens, G. E., & Bormann, F. H. (1972). Nutrient Content of Litter Fall on the Hubbard Brook Experimental Forest, New Hampshire. *Ecology, 53*(5), 769-784. doi: 10.2307/1934293

Gräff, A., Moser, G., & Heiselmayer, P. (2015). Is light interception of understorey species facilitated by light reflection from plant neighbours? *Plant Ecology and Diversity*, *8*(1), 1-12. doi: 10.1080/17550874.2014.898162

Gramss, G., & Bergmann, H. (2008). Role of plants in the vegetative and reproductive growth of saprobic basidiomycetous ground fungi. *Microbial Ecology*, *56*(4), 660-670. doi: 10.1007/s00248-008-9385-8

Grossman, J. J., Cavender-Bares, J., & Hobbie, S. E. (2020). Functional diversity of leaf litter mixtures slows decomposition of labile but not recalcitrant carbon over two years. *Ecological Monographs*, *90*(3), 1-19. doi: 10.1002/ecm.1407

Handa, I. T., Aerts, R., Berendse, F., Berg, M. P., Bruder, A., Butenschoen, O., … Hättenschwiler, S. (2014). Consequences of biodiversity loss for litter decomposition across biomes. *Nature*, *509*(7499), 218-221. doi: 10.1038/nature13247

Hansen, R. A., & Coleman, D. C. (1998). Litter complexity and composition are determinants of the diversity and species composition of oribatid mites (Acari: Oribatida) in litterbags. *Applied Soil Ecology*, *9*(1-3), 17-23. doi: 10.1016/S0929-1393(98)00048-1

Hartley, S. E., & DeGabriel, J. L. (2016). The Ecology of Herbivore-induced Silicon Defences in Grasses. *Functional Ecology*, (30), 1311-1322.

Hättenschwiler, S., & Bretscher, D. (2001). Isopod effects on decomposition of litter produced under elevated CO2, N deposition and different soil types. *Global Change Biology*, *7*(5), 565-579. doi: 10.1046/j.1365-2486.2001.00402.x

Hättenschwiler, Stephan, & Gasser, P. (2005). Soil animals alter plant litter diversity effects on decomposition. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(5), 1519-1524. doi: 10.1073/pnas.0404977102

Hättenschwiler, Stephan, & Jørgensen, H. B. (2010). Carbon quality rather than stoichiometry controls litter decomposition in a tropical rain forest. *Journal of Ecology*, *98*(4), 754-763. doi: 10.1111/j.1365-2745.2010.01671.x

Hättenschwiler, Stephan, Tiunov, A. V., & Scheu, S. (2005). Biodiversity and Litter Decomposition in Terrestrial Ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, *36*(1), 191-218. doi: 10.1146/annurev.ecolsys.36.112904.151932

Hättenschwiler, Stephan, & Vitousek, P. M. (2000). The role of polyphenols in terrestrial ecosystem nutrient cycling. *Trends in Ecology & Evolution*, *15*(6), 238-243. doi: 10.1016/S0169-5347(00)01861-9

He, W., Keyhani, A. B., Ma, Z., Xu, J., Zhang, C., Xu, X., … Wang, P. (2020). Leaf litter lignin degradation in response to understory vegetation removal in a Masson pine plantation. *Land Degradation & Development*, ldr.3766. doi: 10.1002/ldr.3766

He, W., Xu, X., Zhang, C., Ma, Z., Xu, J., Ten, M., … Wang, P. (2020). Understory vegetation removal reduces the incidence of non-additive mass loss during leaf litter decomposition in a subtropical Pinus massoniana plantation. *Plant and Soil*, *446*(1-2), 529-541. doi: 10.1007/s11104-019-04378-2

Hobbie, S. E., Reich, P. B., Oleksyn, J., Ogdahl, M., Zytkowiak, R., Hale, C., & Karolewski, P. (2006). Tree species effects on decomposition and forest floor dynamics in a common garden. *Ecology*, *87*(9), 2288-2297. doi: 10.1890/0012-9658(2006)87[2288:TSEODA]2.0.CO;2

Hoorens, B., Stroetenga, M., & Aerts, R. (2010). Litter mixture interactions at the level of plant functional types are additive. *Ecosystems*, *13*(1), 90-98. doi: 10.1007/s10021-009-9301-1

Horodecki, P., & Jagodziński, A. M. (2017). Tree species effects on litter decomposition in pure stands on afforested post-mining sites. *Forest Ecology and Management*, *406*, 1-11. doi: 10.1016/j.foreco.2017.09.059

Horodecki, P., Nowiński, M., & Jagodziński, A. M. (2019). Advantages of mixed tree stands in restoration of upper soil layers on postmining sites: A five-year leaf litter decomposition experiment. *Land Degradation & Development*, *30*(1), 3-13. doi: 10.1002/ldr.3194

Huang, Z., Clinton, P. W., Baisden, W. T., & Davis, M. R. (2011). Long-term nitrogen additions increased surface soil carbon concentration in a forest plantation despite elevated decomposition. *Soil Biology and Biochemistry*, *43*(2), 302-307. doi: 10.1016/j.soilbio.2010.10.015

Huhta, V. (2007). The role of soil fauna in ecosystems: A historical review. *Pedobiologia*, *50*(6), 489-495. doi: 10.1016/j.pedobi.2006.08.006

Hull, B., Sarah, A., Rien, V., & Martha, M. (2008). Forest Management in the Interface : Reducing Fire Risk. *EDIS*, (6), 1-5. Retrieved from https://journals.flvc.org/edis/article/view/117362.

Hunter, M. D., Adl, S., Pringle, C. M., & Coleman, D. C. (2003). Relative effects of macroinvertebrates and habitat on the chemistry of litter during decomposition. *Pedobiologia*, *47*(2), 101-115. doi: 10.1078/0031-4056-00174

Hyvönen, R., Ågren, G. I., Linder, S., Persson, T., Cotrufo, M. F., Ekblad, A., … Wallin, G. (2007). The likely impact of elevated CO2, nitrogen deposition, increased temperature and management on carbon sequestration in temperate and boreal forest ecosystems: A literature review. *New*

*Phytologist*, *173*(3), 463-480. doi: 10.1111/j.1469-8137.2007.01967.x

Iswaldi, I., Arráez-Román, D., Rodríguez-Medina, I., Beltrán-Debón, R., Joven, J., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2011). Identification of phenolic compounds in aqueous and ethanolic rooibos extracts (Aspalathus linearis) by HPLC-ESI-MS (TOF/IT). *Analytical and Bioanalytical Chemistry*, *400*(10), 3643-3654. doi: 10.1007/s00216-011-4998-z

Jacob, M., Viedenz, K., Polle, A., & Thomas, F. M. (2010). Leaf litter decomposition in temperate deciduous forest stands with a decreasing fraction of beech (Fagus sylvatica). *Oecologia*, *164*(4), 1083-1094. doi: 10.1007/s00442-010-1699-9

Jacob, M., Weland, N., Platner, C., Schaefer, M., Leuschner, C., & Thomas, F. M. (2009). Nutrient release from decomposing leaf litter of temperate deciduous forest trees along a gradient of increasing tree species diversity. *Soil Biology and Biochemistry*, *41*(10), 2122-2130. doi: 10.1016/j.soilbio.2009.07.024

Jagodziński, A. M., Dyderski, M. K., Rawlik, K., & Katna, B. (2016). Seasonal variability of biomass, total leaf area and specific leaf area of forest understory herbs reflects their life strategies. *Forest Ecology and Management*, *374*, 71-81. doi: 10.1016/j.foreco.2016.04.050

Jang, W., Keyes, C. R., & Page-Dumroese, D. (2015). Impact of biomass harvesting on forest soil productivity in the Northern Rocky Mountains. *USDA Forest Service - General Technical Report RMRS-GTR*, *2015*(341). doi: 10.2737/RMRS-GTR-341

Janssens, I. A., Dieleman, W., Luyssaert, S., Subke, J.-A., Reichstein, M., Ceulemans, R., … Law, B. E. (2010). Reduction of forest soil respiration in response to nitrogen deposition. *Nature Geoscience*, *3*(5), 315-322. doi: 10.1038/ngeo844

Jiang, Y., Yin, X., & Wang, F. (2013). The influence of litter mixing on decomposition and soil fauna assemblages in a Pinus koraiensis mixed broad-leaved forest of the Changbai Mountains, China. *European Journal of Soil Biology*, *55*, 28-39. doi: 10.1016/j.ejsobi.2012.11.004

Joly, F.-X., Milcu, A., Scherer-Lorenzen, M., Jean, L.-K., Bussotti, F., Dawud, S. M., … Hättenschwiler, S. (2017). Tree species diversity affects decomposition through modified micro-environmental conditions across European forests. *New Phytologist*, *214*(3), 1281-1293. doi: 10.1111/nph.14452

Jones, G. L., Scullion, J., Worgan, H., & Gwynn-Jones, D. (2019). Litter of the invasive shrub Rhododendron ponticum (Ericaceae) modifies the decomposition rate of native UK woodland litter. *Ecological Indicators*, *107*, 105597. doi: 10.1016/j.ecolind.2019.105597

Jonsson, M., & Wardle, D. A. (2008). Context dependency of litter-mixing effects on decomposition and nutrient release across a long-term chronosequence. *Oikos*, *117*(11), 1674-1682. doi: 10.1111/j.1600-0706.2008.16810.x

Kadavý, J., Adamec, Z., Uherková, B., Kneifl, M., Knott, R., Kučera, A., … Drápela, K. (2019). Growth Response of Sessile Oak and European Hornbeam to Traditional Coppice-with-Standards Management. *Forests, 10*(6), 515. doi: 10.3390/f10060515

Kaneda, S., Angst, Š., & Frouz, J. (2020). Development of Nutrient Uptake by Understory Plant Arrhenatherum elatius and Microbial Biomass during Primary Succession of Forest Soils in Post-Mining Land. *Forests*, *11*(2), 247. doi: 10.3390/f11020247

Keiser, A. D., & Bradford, M. A. (2017). Climate masks decomposer influence in a cross-site litter decomposition study. *Soil Biology and Biochemistry*, *107*, 180-187. doi: 10.1016/j.soilbio.2016.12.022

Kerekes, J., Kaspari, M., Stevenson, B., Nilsson, R. H., Hartmann, M., Amend, A., & Bruns, T. D. (2013). Nutrient enrichment increased species richness of leaf litter fungal assemblages in a tropical forest. *Molecular Ecology*, *22*(10), 2827-2838. doi: 10.1111/mec.12259

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

Knorr, M., Frey, S. D., & Curtis, P. S. (2005). Nitrogen additions and litter decomposition: A meta-analysis. *Ecology, 86*(12), 3252-3257. doi: 10.1890/05-0150

Koide, R. T., & Wu, T. (2003). Ectomycorrhizas and retarded decomposition in a Pinus resinosa plantation. *New Phytologist*, *158*(2), 401-407. doi: 10.1046/j.1469-8137.2003.00732.x

Kolosova, V., Belichenko, O., Rodionova, A., Melnikov, D., & Sõukand, R. (2020). Foraging in Boreal Forest: Wild Food Plants of the Republic of Karelia, NW Russia. *Foods*, *9*(8), 1015. doi: 10.3390/foods9081015

Kooijman, A. M., & Cammeraat, E. (2010). Biological control of beech and hornbeam affects species richness via changes in the organic layer, pH and soil moisture characteristics. *Functional Ecology*, *24*(2), 469-477. doi: 10.1111/j.1365-2435.2009.01640.x

Krishna, M. P., & Mohan, M. (2017). Litter decomposition in forest ecosystems: a review. *Energy, Ecology and Environment*, *2*(4), 236-249. doi: 10.1007/s40974-017-0064-9

Kuzyakov, Y., & Xu, X. (2013). Competition between roots and microorganisms for nitrogen: Mechanisms and ecological relevance. *New Phytologist*, *198*(3), 656-669. doi: 10.1111/nph.12235

Laganière, J., Paré, D., & Bradley, R. L. (2010). How does a tree species influence litter decomposition? Separating the relative contribution of litter quality, litter mixing, and forest floor conditions. *Canadian Journal of Forest Research*, *40*(3), 465-475. doi: 10.1139/X09-208

Landuyt, D., De Lombaerde, E., Perring, M. P., Hertzog, L. R., Ampoorter, E., Maes, S. L., … Verheyen, K. (2019). The functional role of temperate forest understorey vegetation in a changing world. *Global Change Biology*, *25*(11), 3625-3641. doi: 10.1111/gcb.14756

Langenbruch, C., Helfrich, M., & Flessa, H. (2012). Effects of beech (Fagus sylvatica), ash (Fraxinus excelsior) and lime (Tilia spec.) on soil chemical properties in a mixed deciduous forest. *Plant and Soil*, *352*(1-2), 389-403. doi: 10.1007/s11104-011-1004-7

Lefcheck, J. S. (2016). piecewiseSEM: Piecewise structural equation modelling in r for ecology, evolution, and systematics. *Methods in Ecology and Evolution*, *7*(5), 573-579. doi: 10.1111/2041-210X.12512

Lei, L., Xiao, W., Zeng, L., Zhu, J., Huang, Z., Cheng, R., … Li, M. H. (2018). Thinning but not understory removal increased heterotrophic respiration and total soil respiration in Pinus massoniana stands. *Science of the Total Environment*, *621*, 1360-1369. doi: 10.1016/j.scitotenv.2017.10.092

Leppert, K. N., Niklaus, P. A., & Scherer-Lorenzen, M. (2017). Does species richness of subtropical tree leaf litter affect decomposition, nutrient release, transfer and subsequent uptake by plants? *Soil Biology and Biochemistry*, *115*, 44-53. doi: 10.1016/j.soilbio.2017.08.007

Liiri, M., Häsä, M., Haimi, J., & Setälä, H. (2012). History of land-use intensity can modify the relationship between functional complexity of the soil fauna and soil ecosystem services - A microcosm study. *Applied Soil Ecology*, *55*, 53-61. doi: 10.1016/j.apsoil.2011.12.009

Limpens, J., & Berendse, F. (2003). How litter quality affects mass loss and N loss from decomposing Sphagnum. *Oikos*, *103*(3), 537-547. doi: 10.1034/j.1600-0706.2003.12707.x

Lin, D., Xia, J., & Wan, S. (2010). Climate warming and biomass accumulation of terrestrial plants: A meta-analysis. *New Phytologist*, *188*(1), 187-198. doi: 10.1111/j.1469-8137.2010.03347.x

Liu, B. C., Liu, W., & Peng, S. W. (2005). Study of heat and moisture transfer in soil with a dry surface layer. *International Journal of Heat and Mass Transfer*, *48*(21-22), 4579-4589. doi: 10.1016/j.ijheatmasstransfer.2005.06.004

Liu, J., Liu, X., Song, Q., Compson, Z. G., LeRoy, C. J., Luan, F., … Yang, Q. (2020). Synergistic effects: a common theme in mixed-species litter decomposition. *New Phytologist*, *227*(3), 757-

765. doi: 10.1111/nph.16556

Liu, P., Huang, J., Han, X., Sun, O. J., & Zhou, Z. (2006). Differential responses of litter decomposition to increased soil nutrients and water between two contrasting grassland plant species of Inner Mongolia, China. *Applied Soil Ecology*, *34*(2-3), 266-275. doi: 10.1016/j.apsoil.2005.12.009

Liu, X., Ming, L. L., Nizzetto, L., Borgå, K., Larssen, T., Zheng, Q., … Zhang, G. (2013). Critical evaluation of a new passive exchange-meter for assessing multimedia fate of persistent organic pollutants at the air-soil interface. *Environmental Pollution*, *181*, 144-150. doi: 10.1016/j.envpol.2013.06.020

Lorimer, C. G., Chapman, J. W., & Lambert, W. D. (1994). Tall Understorey Vegetation as a Factor in the Poor Development of Oak Seedlings Beneath Mature Stands. *The Journal of Ecology*, *82*(2), 227. doi: 10.2307/2261291

Lukianchuk, A., Khropot, O., Konechnyi, Y., Konechna, R., & Novikov, V. (2017). Wood anemone. Anemone Nemorosa L. Analytical review. *ScienceRise: Pharmaceutical Science*, *3*(7), 34-38. doi: 10.15587/2519-4852.2017.104438

Lummer, D., Scheu, S., & Butenschoen, O. (2012). Connecting litter quality, microbial community and nitrogen transfer mechanisms in decomposing litter mixtures. *Oikos*, *121*(10), 1649-1655. doi: 10.1111/j.1600-0706.2011.20073.x

Luo, R., Fan, J., Wang, W., Luo, J., Kuzyakov, Y., He, J.-S., … Ding, W. (2019). Nitrogen and phosphorus enrichment accelerates soil organic carbon loss in alpine grassland on the Qinghai-Tibetan Plateau. *Science of The Total Environment*, *650*, 303-312. doi: 10.1016/j.scitotenv.2018.09.038

Madritch, M. D., & Cardinale, B. J. (2007). Impacts of tree species diversity on litter decomposition in northern temperate forests of Wisconsin, USA: A multi-site experiment along a latitudinal gradient. *Plant and Soil*, *292*(1-2), 147-159. doi: 10.1007/s11104-007-9209-5

Maes, S. L., Blondeel, H., Perring, M. P., Depauw, L., Brūmelis, G., Brunet, J., … Verheyen, K. (2019). Litter quality, land-use history, and nitrogen deposition effects on topsoil conditions across European temperate deciduous forests. *Forest Ecology and Management*, *433*, 405-418. doi: 10.1016/j.foreco.2018.10.056

Maes, S. L., De Frenne, P., Brunet, J., de la Peña, E., Chabrerie, O., Cousins, S. A. O., … Verheyen, K. (2014). Effects of enhanced nitrogen inputs and climate warming on a forest understorey plant assessed by transplant experiments along a latitudinal gradient. *Plant Ecology*, *215*(8), 899-910.

doi: 10.1007/s11258-014-0341-z

Makkonen, M., Berg, M. P., van Logtestijn, R. S. P., van Hal, J. R., & Aerts, R. (2013). Do physical plant litter traits explain non-additivity in litter mixtures? A test of the improved microenvironmental conditions theory. *Oikos*, *122*(7), 987-997. doi: 10.1111/j.1600-0706.2012.20750.x

Manzoni, S., Jackson, R. B., Trofymow, J. A., & Porporato, A. (2008). The global stoichiometry of litter nitrogen mineralization. *Science*, *321*(5889), 684-686. doi: 10.1126/science.1159792

Marchin, R. M., Salk, C. F., Hoffmann, W. A., & Dunn, R. R. (2015). Temperature alone does not explain phenological variation of diverse temperate plants under experimental warming. *Global Change Biology*, *21*(8), 3138-3151. doi: 10.1111/gcb.12919

Matsushima, M., & Chang, S. X. (2007). Effects of understory removal, N fertilization, and litter layer removal on soil N cycling in a 13-year-old white spruce plantation infested with Canada bluejoint grass. *Plant and Soil*, *292*(1-2), 243-258. doi: 10.1007/s11104-007-9220-x

Matthews, E. (1997). Global litter production, pools, and turnover times: Estimates from measurement data and regression models. *Journal of Geophysical Research Atmospheres*, *102*(15), 18771-18800. doi: 10.1029/97jd02956

McArthur, J. V., Aho, J. M., Rader, R. B., & Mills, G. L. (1994). Interspecific Leaf Interactions during Decomposition in Aquatic and Floodplain Ecosystems. *Journal of the North American Benthological Society*, *13*(1), 57-67. doi: 10.2307/1467265

Mcguire, K. L., & Treseder, K. K. (2010). Microbial communities and their relevance for ecosystem models : Decomposition as a case study. *Soil Biology and Biochemistry*, *42*(4), 529-535. doi: 10.1016/j.soilbio.2009.11.016

Melguizo-Ruiz, N., Jiménez-Navarro, G., De Mas, E., Pato, J., Scheu, S., Austin, A. T., … Moya-Laraño, J. (2020). Field exclusion of large soil predators impacts lower trophic levels and decreases leaf-litter decomposition in dry forests. *Journal of Animal Ecology*, *89*(2), 334-346. doi: 10.1111/1365-2656.13101

Melillo, J. M., Aber, J. D., & Muratore, J. F. (1982). Nitrogen and Lignin Control of Hardwood Leaf Litter Decomposition Dynamics. *Ecology*, *63*(3), 621-626. doi: 10.2307/1936780

Milcu, A., Partsch, S., Langel, R., & Scheu, S. (2006). The response of decomposers (earthworms, springtails and microorganisms) to variations in species and functional group diversity of plants. *Oikos*, *112*(3), 513-524. doi: 10.1111/j.0030-1299.2006.14292.x

Mitchell, R. J., Keith, A. M., Potts, J. M., Ross, J., Reid, E., & Dawson, L. A. (2012). Overstory and understory vegetation interact to alter soil community composition and activity. *Plant and Soil*, *352*(1-2), 65-84. doi: 10.1007/s11104-011-0980-y

Mitchell, S. R., Harmon, M. E., & O'Connell, K. E. B. (2009). Forest fuel reduction alters fire severity and long-term carbon storage in three Pacific Northwest ecosystems. *Ecological Applications*, *19*(3), 643-655. doi: 10.1890/08-0501.1

Mølgaard, P. (1986). Food plant preferences by slugs and snails: A simple method to evaluate the relative palatability of the food plants. *Biochemical Systematics and Ecology*, *14*(1), 113-121. doi: 10.1016/0305-1978(86)90095-5

Moore, T. R., Trofymow, J. A., Prescott, C. E., & Titus, B. D. (2011). Nature and nurture in the dynamics of C, N and P during litter decomposition in Canadian forests. *Plant and Soil*, *339*(1), 163-175. doi: 10.1007/s11104-010-0563-3

Murphy, K. L., Klopatek, J. M., & Klopatek, C. C. (1998). The Effects of Litter Quality and Climate on Decomposition along an Elevational Gradient. *Ecological Applications*, *8*(4), 1061-1071. Retrieved from http://www.jstor.org/stable/2640961

Muukkonen, P., Mäkipää, R., Laiho, R., Minkkinen, K., Vasander, H., & Finér, L. (2006). Relationship between biomass and percentage cover in understorey vegetation of boreal coniferous forests. *Silva Fennica*, *40*(2), 231-245. doi: 10.14214/sf.340

Myers-Smith, I. H., Forbes, B. C., Wilmking, M., Hallinger, M., Lantz, T., Blok, D., … Hik, D. S. (2011). Shrub expansion in tundra ecosystems: dynamics, impacts and research priorities. *Environmental Research Letters*, *6*(4), 045509. doi: 10.1088/1748-9326/6/4/045509

Myers-Smith, I. H., & Hik, D. S. (2013). Shrub canopies influence soil temperatures but not nutrient dynamics: An experimental test of tundra snow-shrub interactions. *Ecology and Evolution*, *3*(11), 3683-3700. doi: 10.1002/ece3.710

Nambiar, E. K. S., & Sands, R. (1993). Competition for water and nutrients in forests. *Canadian Journal of Forest Research*, *23*(10), 1955-1968. doi: 10.16309/j.cnki.issn.1007-1776.2003.03.004

Niinemets, Ü. (2010). A review of light interception in plant stands from leaf to canopy in different plant functional types and in species with varying shade tolerance. *Ecological Research*, *25*(4), 693-714. doi: 10.1007/s11284-010-0712-4

Ochoa-Hueso, R., Delgado-Baquerizo, M., An King, P. T., Benham, M., Arca, V., & Power, S. A. (2019). Ecosystem type and resource quality are more important than global change drivers in regulating early stages of litter decomposition. *Soil Biology and Biochemistry*, *129*(2018), 144-152. doi:

10.1016/j.soilbio.2018.11.009

Økland, T., Rydgren, K., Økland, R. H., Storaunet, K. O., & Rolstad, J. (2003). Variation in environmental conditions, understorey species number, abundance and composition among natural and managed Picea abies forest stands. *Forest Ecology and Management*, *177*(1-3), 17-37. doi: 10.1016/S0378-1127(02)00331-6

Oksanen, J., F. Guillaume Blanchet, R. K., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson, G. L., … M. Henry H. Stevens, H. W. (2019). Package 'vegan'. *R Package Version 3.4.0*.

Olson, J. S. (1963). Energy storage and the balance of producers and decomposers in ecological systems. *Ecology*, *44*(2), 322-331. doi: 10.2307/1932179

Ooms, A., Dias, A. T. C., van Oosten, A. R., Cornelissen, J. H. C., Ellers, J., & Berg, M. P. (2020). Species richness and functional diversity of isopod communities vary across an urbanisation gradient, but the direction and strength depend on soil type. *Soil Biology and Biochemistry*, *148*, 107851. doi: 10.1016/j.soilbio.2020.107851

Özkan, U., & Gökbulak, F. (2017). Effect of vegetation change from forest to herbaceous vegetation cover on soil moisture and temperature regimes and soil water chemistry. *Catena*, *149*, 158-166. doi: 10.1016/j.catena.2016.09.017

Pan, Y., Birdsey, R. A., Fang, J., Houghton, R., Kauppi, P. E., Kurz, W. A., … Hayes, D. (2011). A large and persistent carbon sink in the world's forests. *Science, 333*(6045), 988-993. doi: 10.1126/science.1201609

Parton, W., Silver, W. L., Burke, I. C., Grassens, L., Harmon, M. E., Currie, W. S., … Fasth, B. (2007). Global-scale similarities in nitrogen release patterns during long-term decomposition. *Science*, *315*(5810), 361-364. doi: 10.1126/science.1134853

Pecot, S. D., Mitchell, R. J., Palik, B. J., Moser, E. B., & Hiers, J. K. (2007). Competitive responses of seedlings and understory plants in longleaf pine woodlands: Separating canopy influences above and below ground. *Canadian Journal of Forest Research*, *37*(3), 634-648. doi: 10.1139/X06-247

Pérez-Suárez, M., Arredondo-Moreno, J. T., & Huber-Sannwald, E. (2012). Early stage of single and mixed leaf-litter decomposition in semiarid forest pine-oak: The role of rainfall and microsite. *Biogeochemistry*, *108*(1-3), 245-258. doi: 10.1007/s10533-011-9594-y

Pérez Harguindeguy, N., Blundo, C. M., Gurvich, D. E., Díaz, S., & Cuevas, E. (2008). More than the sum of its parts? Assessing litter heterogeneity effects on the decomposition of litter mixtures through leaf chemistry. *Plant and Soil*, *303*(1-2), 151-159. doi: 10.1007/s11104-007-9495-y

Perring, M. P., Bernhardt-Römermann, M., Baeten, L., Midolo, G., Blondeel, H., Depauw, L., … Verheyen, K. (2018). Global environmental change effects on plant community composition trajectories depend upon management legacies. *Global Change Biology*, *24*(4), 1722-1740. doi: 10.1111/gcb.14030

Petraglia, A., Cacciatori, C., Chelli, S., Fenu, G., Calderisi, G., Gargano, D., … Carbognani, M. (2019). Litter decomposition: effects of temperature driven by soil moisture and vegetation type. *Plant and Soil, 435*, 187-200. doi: 10.1007/s11104-018-3889-x

Pontégnie, M., Du Bus De Warnaffe, G., & Lebrun, P. (2005). Impacts of silvicultural practices on the structure of hemi-edaphic macrofauna community. *Pedobiologia*, *49*(3), 199-210. doi: 10.1016/j.pedobi.2004.09.005

Portillo-Estrada, M., Pihlatie, M., Korhonen, J. F. J., Levula, J., Frumau, A. K. F., Ibrom, A., … Niinemets, Ü. (2016). Climatic controls on leaf litter decomposition across European forests and grasslands revealed by reciprocal litter transplantation experiments. *Biogeosciences*, *13*(5), 1621-1633. doi: 10.5194/bg-13-1621-2016

Prescott, C. E. (2002). The influence of the forest canopy on nutrient cycling. *Tree Physiology*, *22*(15-16), 1193-1200. doi: 10.1093/treephys/22.15-16.1193

Prescott, C. E. (2010). Litter decomposition: What controls it and how can we alter it to sequester more carbon in forest soils? *Biogeochemistry, 101*(1), 133-149. doi: 10.1007/s10533-010-9439-0

Quadros, A. F., Zimmer, M., Araujo, P. B., & Kray, J. G. (2014). Litter traits and palatability to detritivores: a case study across bio-geographical boundaries. *Nauplius*, *22*(2), 103-111. doi: 10.1590/s0104-64972014000200004

R Core Team. (2018). R: A language and environment for statistical computin. In *URL*.

Rahman, M. M., Castagneyrol, B., Verheyen, K., Jactel, H., & Carnol, M. (2018). Can tree species richness attenuate the effect of drought on organic matter decomposition and stabilization in young plantation forests? *Acta Oecologica*, *93*, 30-40. doi: 10.1016/j.actao.2018.10.008

Rajapaksha, N. S. S., Butt, K. R., Vanguelova, E. I., & Moffat, A. J. (2013a). Earthworm selection of Short Rotation Forestry leaf litter assessed through preference testing and direct observation. *Soil Biology and Biochemistry*, *67*, 12-19. doi: 10.1016/j.soilbio.2013.08.006

Rajapaksha, N. S. S., Butt, K. R., Vanguelova, E. I., & Moffat, A. J. (2013b). Effects of Short Rotation Forestry on earthworm community development in the UK. *Forest Ecology and Management*, *309*, 96-104. doi: 10.1016/j.foreco.2013.04.004

Reis, F., Nascimento, E., Castro, H., Canhoto, C., Gonçalves, A. L., Simões, S., … Martins da Silva, P. (2018). Land management impacts on the feeding preferences of the woodlouse Porcellio dilatatus (Isopoda: Oniscidea) via changes in plant litter quality. *Applied Soil Ecology*, *132*, 45-52. doi: 10.1016/j.apsoil.2018.08.018

Ritter, E., & Vesterdal, L. (2006). Gap formation in Danish beech (Fagus sylvatica) forests of low management intensity: Soil moisture and nitrate in soil solution. *European Journal of Forest Research*, *125*(2), 139-150. doi: 10.1007/s10342-005-0077-3

Riutta, T., Slade, E. M., Bebber, D. P., Taylor, M. E., Malhi, Y., Riordan, P., … Morecroft, M. D. (2012). Experimental evidence for the interacting effects of forest edge, moisture and soil macrofauna on leaf litter decomposition. *Soil Biology and Biochemistry*, *49*, 124-131. doi: 10.1016/j.soilbio.2012.02.028

Rosenberg, O., & Jacobson, S. (2004). Effects of repeated slash removal in thinned stands on soil chemistry and understorey vegetation. *Silva Fennica*, *38*(2), 133-142. doi: 10.14214/sf.423

Rouifed, S., Handa, I. T., David, J. F., & Hättenschwiler, S. (2010). The importance of biotic factors in predicting global change effects on decomposition of temperate forest leaf litter. *Oecologia*, *163*(1), 247-256. doi: 10.1007/s00442-009-1528-1

Royer, P. D., Cobb, N. S., Clifford, M. J., Huang, C. Y., Breshears, D. D., Adams, H. D., & Villegas, J. C. (2011). Extreme climatic event-triggered overstorey vegetation loss increases understorey solar input regionally: Primary and secondary ecological implications. *Journal of Ecology*, *99*(3), 714-723. doi: 10.1111/j.1365-2745.2011.01804.x

Santonja, M., Fernandez, C., Proffit, M., Gers, C., Gauquelin, T., Reiter, I. M., … Baldy, V. (2017). Plant litter mixture partly mitigates the negative effects of extended drought on soil biota and litter decomposition in a Mediterranean oak forest. *Journal of Ecology*, *105*(3), 801-815. doi: 10.1111/1365-2745.12711

Santos, F. M., Balieiro, F. de C., Fontes, M. A., & Chaer, G. M. (2018). Understanding the enhanced litter decomposition of mixed-species plantations of Eucalyptus and Acacia mangium. *Plant and Soil*, *423*(1-2), 141-155. doi: 10.1007/s11104-017-3491-7

Sariyildiz, T., & Anderson, J. M. (2003). Interactions between litter quality, decomposition and soil fertility: A laboratory study. *Soil Biology and Biochemistry*, *35*(3), 391-399. doi: 10.1016/S0038-0717(02)00290-0

Saura-Mas, S., Estiarte, M., Peñuelas, J., & Lloret, F. (2012). Effects of climate change on leaf litter decomposition across post-fire plant regenerative groups. *Environmental and Experimental Botany*, *77*, 274-282. doi: 10.1016/j.envexpbot.2011.11.014

Sayer, E. J. (2006). Using experimental manipulation to assess the roles of leaf litter in the functioning of forest ecosystems. *Biological Reviews of the Cambridge Philosophical Society*, *81*(1), 1-31. doi: 10.1017/S1464793105006846

Schädler, M., Jung, G., Auge, H., & Brandl, R. (2003). Palatability, decomposition and insect herbivory: patterns in a successional old-field plant community. *Oikos*, *103*(1), 121-132. doi: 10.1034/j.1600-0706.2003.12659.x

Scheibe, A., Steffens, C., Seven, J., Jacob, A., Hertel, D., Leuschner, C., & Gleixner, G. (2015). Effects of tree identity dominate over tree diversity on the soil microbial community structure. *Soil Biology and Biochemistry*, *81*, 219-227. doi: 10.1016/j.soilbio.2014.11.020

Schelfhout, S., Mertens, J., Verheyen, K., Vesterdal, L., Baeten, L., Muys, B., & De Schrijver, A. (2017). Tree species identity shapes earthworm communities. *Forests*, *8*(3), 85. doi: 10.3390/f8030085

Schimel, J. P., & Hättenschwiler, S. (2007). Nitrogen transfer between decomposing leaves of different N status. *Soil Biology and Biochemistry*, *39*(7), 1428-1436. doi: 10.1016/j.soilbio.2006.12.037

Schon, N. L., Mackay, A. D., Minor, M. A., Yeates, G. W., & Hedley, M. J. (2008). Soil fauna in grazed New Zealand hill country pastures at two management intensities. *Applied Soil Ecology*, *40*(2), 218-228. doi: 10.1016/j.apsoil.2008.04.007

Schweitzer, J. A., Bailey, J. K., Hart, S. C., & Whitham, T. G. (2005). Nonadditive effects of mixing cottonwood genotypes on litter decomposition and nutrient dynamics. *Ecology*, *86*(10), 2834-2840. doi: 10.1890/04-1955

Setiawan, N. N., Vanhellemont, M., De Schrijver, A., Schelfhout, S., Baeten, L., & Verheyen, K. (2016). Mixing effects on litter decomposition rates in a young tree diversity experiment. *Acta Oecologica*, *70*, 79-86. doi: 10.1016/j.actao.2015.12.003

Shannon, C. E. (1963). A mathematical theory of communication. In *University of Illinois Press, Urbana*.

Shaw, M. R., & Harte, J. (2001). Control of litter decomposition in a subalpine meadow-sagebrush steppe ecotone under climate change. *Ecological Applications*, *11*(4), 1206-1223. doi: 10.1890/1051-0761(2001)011[1206:COLDIA]2.0.CO;2

Shipley, B. (2009). Confirmatory path analysis in a generalized multilevel context. *Ecology*, *90*(2), 363-368. doi: 10.1890/08-1034.1

Shirreffs, D. A. (1985). Anemone Nemorosa L. *Journal of Ecology*, *73*(3), 1005-1020. doi:

10.2307/2260164

Sierra, C. A., Malghani, S., & Loescher, H. W. (2017). Interactions among temperature, moisture, and oxygen concentrations in controlling decomposition rates in a boreal forest soil. *Biogeosciences, 14*(3), 703-710. doi: 10.5194/bg-14-703-2017

Slade, E. M., & Riutta, T. (2012). Interacting effects of leaf litter species and macrofauna on decomposition in different litter environments. *Basic and Applied Ecology*, *13*(5), 423-431. doi: 10.1016/j.baae.2012.06.008

Smith, A. P., Marín-Spiotta, E., & Balser, T. (2015). Successional and seasonal variations in soil and litter microbial community structure and function during tropical postagricultural forest regeneration: A multiyear study. *Global Change Biology*, *21*(9), 3532-3547. doi: 10.1111/gcb.12947

Smith, S. W., Speed, J. D. M., Bukombe, J., Hassan, S. N., Lyamuya, R. D., Mtweve, P. J., … Graae, B. J. (2019). Litter type and termites regulate root decomposition across contrasting savanna land-uses. *Oikos*, *128*(4), 596-607. doi: 10.1111/oik.05697

Sollins, P., Homann, P., & Caldwell, B. A. (1996). Stabilization and destabilization of soil organic matter. *Geoderma, 74*(1-2), 65-105. doi: 10.1016/S0016-7061(96)00036-5

Špaldoňová, A., & Frouz, J. (2014). The role of Armadillidium vulgare (Isopoda: Oniscidea) in litter decomposition and soil organic matter stabilization. *Applied Soil Ecology*, *83*, 186-192. doi: 10.1016/j.apsoil.2014.04.012

Staelens, J., De Schrijver, A., Verheyen, K., & Verhoest, N. E. C. (2006). Spatial variability and temporal stability of throughfall water under a dominant beech (Fagus sylvatica L.) tree in relationship to canopy cover. *Journal of Hydrology, 330*(3-4), 651-662. doi: 10.1016/j.jhydrol.2006.04.032

Stanek, M., Piechnik, Ł., & Stefanowicz, A. M. (2020). Invasive red oak (Quercus rubra L.) modifies soil physicochemical properties and forest understory vegetation. *Forest Ecology and Management*, *472*, 118253. doi: 10.1016/j.foreco.2020.118253

Swift, M. J., Heal, O. W., & Anderson, J. M. (1979). Decomposition in Terrestrial Ecosystems. In *Blackwell Scientific Publications, Oxford*.

Tardif, A., & Shipley, B. (2015). The relationship between functional dispersion of mixed-species leaf litter mixtures and species' interactions during decomposition. *Oikos*, *124*(8), 1050-1057. doi: 10.1111/oik.01686

Tardif, A., Shipley, B., Bloor, J. M. G., & Soussana, J. F. (2014). Can the biomass-ratio hypothesis predict mixed-species litter decomposition along a climatic gradient? *Annals of Botany*, *113*(5), 843-850. doi: 10.1093/aob/mct304

Thiessen, S., Gleixner, G., Wutzler, T., & Reichstein, M. (2013). Both priming and temperature sensitivity of soil organic matter decomposition depend on microbial biomass - An incubation study. *Soil Biology and Biochemistry*, *57*, 739-748. doi: 10.1016/j.soilbio.2012.10.029

Thirukkumaran, C. M., & Parkinson, D. (2000). Microbial respiration, biomass, metabolic quotient and litter decomposition in a lodgepole pine forest floor amended with nitrogen and phosphorous fertilizers. *Soil Biology and Biochemistry*, *32*(1), 59-66. doi: 10.1016/S0038-0717(99)00129-7

Thrippleton, T., Bugmann, H., Kramer-Priewasser, K., & Snell, R. S. (2016). Herbaceous Understorey: An Overlooked Player in Forest Landscape Dynamics? *Ecosystems*, *19*(7), 1240-1254. doi: 10.1007/s10021-016-9999-5

Tranvik, L., & Eijsackers, H. (1989). International association for ecology on the advantage of Folsomia fimetarioides over Isotomiella minor ( Collembola ) in a Metal Polluted Soil. *International Association for Ecology*, *80*(2), 195-200. doi: 10.1007/BF00380150

Treseder, K. K. (2008). Nitrogen additions and microbial biomass: A meta-analysis of ecosystem studies. *Ecology Letters*, Vol. 11, pp. 1111-1120. doi: 10.1111/j.1461-0248.2008.01230.x

Trofymow, J. A., Moore, T. R., Titus, B., Prescott, C., Morrison, I., Siltanen, M., … Visser, S. (2002). Rates of litter decomposition over 6 years in Canadian forests: Influence of litter quality and climate. *Canadian Journal of Forest Research*, *32*(5), 789-804. doi: 10.1139/x01-117

Trogisch, S., He, J. S., Hector, A., & Scherer-Lorenzen, M. (2016). Impact of species diversity, stand age and environmental factors on leaf litter decomposition in subtropical forests in China. *Plant and Soil*, *400*(1-2), 337-350. doi: 10.1007/s11104-015-2737-5

Van Groenigen, K. J., Qi, X., Osenberg, C. W., Luo, Y., & Hungate, B. A. (2014). Faster decomposition under increased atmospheric CO2 limits soil carbon storage. *Science*, *344*(6183), 508-509. doi: 10.1126/science.1249534

van Loon, M. P., Schieving, F., Rietkerk, M., Dekker, S. C., Sterck, F., & Anten, N. P. R. (2014). How light competition between plants affects their response to climate change. *New Phytologist*, *203*(4), 1253-1265. doi: 10.1111/nph.12865

Vanneste, T., Govaert, S., Spicher, F., Brunet, J., Cousins, S. A. O., Decocq, G., … De Frenne, P. (2020). Contrasting microclimates among hedgerows and woodlands across temperate Europe. *Agricultural and Forest Meteorology*, *281*, 107818. doi: 10.1016/j.agrformet.2019.107818

Verheyen, K., Baeten, L., De Frenne, P., Bernhardt-Römermann, M., Brunet, J., Cornelis, J., …

Verstraeten, G. (2012). Driving factors behind the eutrophication signal in understorey plant communities of deciduous temperate forests. *Journal of Ecology*, *100*(2), 352-365. doi: 10.1111/j.1365-2745.2011.01928.x

Vesterdal, L. (1999). Influence of soil type on mass loss and nutrient release from decomposing foliage litter of beech and Norway spruce. *Canadian Journal of Forest Research*, *29*(1), 95-105. doi: 10.1139/x98-182

Vesterdal, L., Schmidt, I. K., Callesen, I., Nilsson, L. O., & Gundersen, P. (2008). Carbon and nitrogen in forest floor and mineral soil under six common European tree species. *Forest Ecology and Management*, *255*(1), 35-48. doi: 10.1016/j.foreco.2007.08.015

Vild, O., Šipoš, J., Szabó, P., Macek, M., Chudomelová, M., Kopecký, M., … Hédl, R. (2018). Legacy of historical litter raking in temperate forest plant communities. *Journal of Vegetation Science*, *29*(4), 596-606. doi: 10.1111/jvs.12642

Vitasse, Y., François, C., Delpierre, N., Dufrêne, E., Kremer, A., Chuine, I., & Delzon, S. (2011). Assessing the effects of climate change on the phenology of European temperate trees. *Agricultural and Forest Meteorology*, *151*(7), 969-980. doi: 10.1016/j.agrformet.2011.03.003

Vivanco, L., & Austin, A. T. (2008). Tree species identity alters forest litter decomposition through long-term plant and soil interactions in Patagonia, Argentina. *Journal of Ecology, 96*(4), 727-736. doi: 10.1111/j.1365-2745.2008.01393.x

Vos, V. C. A., van Ruijven, J., Berg, M. P., Peeters, E. T. H. M., & Berendse, F. (2011). Macro-detritivore identity drives leaf litter diversity effects. *Oikos*, *120*(7), 1092-1098. doi: 10.1111/j.1600-0706.2010.18650.x

Vos, V. C. A., van Ruijven, J., Berg, M. P., Peeters, E. T. H. M., & Berendse, F. (2013). Leaf litter quality drives litter mixing effects through complementary resource use among detritivores. *Oecologia*, *173*(1), 269-280. doi: 10.1007/s00442-012-2588-1

Wahren, C. H. A., Walker, M. D., & Bret-Harte, M. S. (2005). Vegetation responses in Alaskan arctic tundra after 8 years of a summer warming and winter snow manipulation experiment. *Global Change Biology*, *11*(4), 537-552. doi: 10.1111/j.1365-2486.2005.00927.x

Walker, D. A. (2000). Hierarchical subdivision of Artic tundra based on vegetation response to climate, parent material and topography. *Global Change Biology*, *6*(SUPPLEMENT 1), 19-34. doi: 10.1046/j.1365-2486.2000.06010.x

Wall, D. H., Bradford, M. A., St. John, M. G., Trofymow, J. A., Behan-Pelletier, V., Bignell, D. E., …

Zou, X. (2008). Global decomposition experiment shows soil animal impacts on decomposition are climate-dependent. *Global Change Biology*, *14*(11), 2661-2677. doi: 10.1111/j.1365-2486.2008.01672.x

Wang, B., Blondeel, H., Baeten, L., Djukic, I., De Lombaerde, E., & Verheyen, K. (2019). Direct and understorey-mediated indirect effects of human-induced environmental changes on litter decomposition in temperate forest. *Soil Biology and Biochemistry*, *138*, 107579. doi: 10.1016/j.soilbio.2019.107579

Wang, B., Verheyen, K., Baeten, L., & De Smedt, P. (2021). Herb litter mediates tree litter decomposition and soil fauna composition. *Soil Biology and Biochemistry*, *152*, 108063. doi: 10.1016/j.soilbio.2020.108063

Wang, B., Wu, F., Xiao, S., Yang, W., Justine, M. F., He, J., & Tan, B. (2016). Effect of succession gaps on the understory water-holding capacity in an over-mature alpine forest at the upper reaches of the Yangtze River. *Hydrological Processes*, *30*(5), 692-703. doi: 10.1002/hyp.10613

Wang, F., Zou, B., Li, H., & Li, Z. (2014). The effect of understory removal on microclimate and soil properties in two subtropical lumber plantations. *Journal of Forest Research*, *19*(1), 238-243. doi: 10.1007/s10310-013-0395-0

Wang, G., Huang, W., Mayes, M. A., Liu, X., Zhang, D., Zhang, Q., … Zhou, G. (2019). Soil moisture drives microbial controls on carbon decomposition in two subtropical forests. *Soil Biology and Biochemistry*, *130*, 185-194. doi: 10.1016/j.soilbio.2018.12.017

Wang, Y. P., Law, R. M., & Pak, B. (2010). A global model of carbon, nitrogen and phosphorus cycles for the terrestrial biosphere. *Biogeosciences*, *7*(7), 2261-2282. doi: 10.5194/bg-7-2261-2010

Wardle, D. A., Nilsson, M. C., Zackrisson, O., & Gallet, C. (2003). Determinants of litter mixing effects in a Swedish boreal forest. *Soil Biology and Biochemistry*, *35*(6), 827-835. doi: 10.1016/S0038-0717(03)00118-4

Wardle, D. A., Yeates, G. W., Barker, G. M., & Bonner, K. I. (2006). The influence of plant litter diversity on decomposer abundance and diversity. *Soil Biology and Biochemistry*, *38*(5), 1052-1062. doi: 10.1016/j.soilbio.2005.09.003

Welch, N. T., Belmont, J. M., & Randolph, J. C. (2007). Summer ground layer biomass and nutrient contribution to above-ground litter in an Indiana temperate deciduous forest. *The American Midland Naturalist*, *157*(1), 11-26. doi: 10.1674/0003-0031(2007)157[11:SGLBAN]2.0.CO;2

Wickham, H. (2016). ggplot2: Elegant graphics for data analysis. In *Springer-Verlag*. doi:

10.1080/15366367.2019.1565254

Wu, F., Peng, C., Yang, W., Zhang, J., Han, Y., & Mao, T. (2014). Admixture of alder (Alnus formosana) litter can improve the decomposition of eucalyptus (Eucalyptus grandis) litter. *Soil Biology and Biochemistry*, *73*, 115-121. doi: 10.1016/j.soilbio.2014.02.018

Wu, Jian ping, Liu, Z. feng, Wang, X. ling, Sun, Y. xin, Zhou, L. xia, Lin, Y. biao, & Fu, S. lei. (2011). Effects of understory removal and tree girdling on soil microbial community composition and litter decomposition in two Eucalyptus plantations in South China. *Functional Ecology*, *25*(4), 921-931. doi: 10.1111/j.1365-2435.2011.01845.x

Wu, Jianping, Liu, W., Zhang, W., Shao, Y., Duan, H., Chen, B., … Fan, H. (2019). Long-term nitrogen addition changes soil microbial community and litter decomposition rate in a subtropical forest. *Applied Soil Ecology*, *142*, 43-51. doi: 10.1016/j.apsoil.2019.05.014

Xiao, W., Chen, C., Chen, H. Y. H., & Chen, X. (2020). Functional and phylogenetic diversity promote litter decomposition across terrestrial ecosystems. *Global Ecology and Biogeography*, *29*(12), 2261-2272. doi: 10.1111/geb.13181

Xiong, Y. mei, Xia, H. ping, Li, Z., Cai, X., & Fu, S. lei. (2008). Impacts of litter and understory removal on soil properties in a subtropical Acacia mangium plantation in China. *Plant and Soil*, *304*(1-2), 179-188. doi: 10.1007/s11104-007-9536-6

Xu, Xiaoniu, & Hirata, E. (2005). Decomposition patterns of leaf litter of seven common canopy species in a subtropical forest: N and P dynamics. *Plant and Soil*, *273*(1-2), 279-289. doi: 10.1007/s11104-004-8069-5

Xu, Xuan, Sun, Y., Sun, J., Cao, P., Wang, Y., Chen, H. Y. H., … Ruan, H. (2020). Cellulose dominantly affects soil fauna in the decomposition of forest litter: A meta-analysis. *Geoderma*, *378*, 114620. doi: 10.1016/j.geoderma.2020.114620

Xu, Z., Wan, C., Xiong, P., Tang, Z., Hu, R., Cao, G., & Liu, Q. (2010). Initial responses of soil CO2 efflux and C, N pools to experimental warming in two contrasting forest ecosystems, Eastern Tibetan Plateau, China. *Plant and Soil*, *336*(1), 183-195. doi: 10.1007/s11104-010-0461-8

Xu, Z., Zhao, C., Yin, H., & Liu, Q. (2015). Warming and forest management interactively affect the decomposition of subalpine forests on the eastern Tibetan Plateau: A four-year experiment. *Geoderma*, *239-240*, 223-228. doi: 10.1016/j.geoderma.2014.10.018

Yang, N., Butenschoen, O., Rana, R., Köhler, L., Hertel, D., Leuschner, C., … Pena, R. (2019). Leaf litter species identity influences biochemical composition of ectomycorrhizal fungi. *Mycorrhiza*, *29*(2), 85-96. doi: 10.1007/s00572-018-0876-2

Yin, R., Eisenhauer, N., Auge, H., Purahong, W., Schmidt, A., & Schädler, M. (2019). Additive effects of experimental climate change and land use on faunal contribution to litter decomposition. *Soil Biology and Biochemistry*, *131*, 141-148. doi: 10.1016/j.soilbio.2019.01.009

Zellweger, F., Coomes, D., Lenoir, J., Depauw, L., Maes, S. L., Wulf, M., … De Frenne, P. (2019). Seasonal drivers of understorey temperature buffering in temperate deciduous forests across Europe. *Global Ecology and Biogeography*, *28*(12), 1774-1786. doi: 10.1111/geb.12991

Zellweger, F., De Frenne, P., Lenoir, J., Vangansbeke, P., Verheyen, K., Bernhardt-Römermann, M., … Coomes, D. (2020). Forest microclimate dynamics drive plant responses to warming. *Science*, *368*(6492), 772-775. doi: 10.1126/science.aba6880

Zhao, J., Wan, S., Fu, S., Wang, X., Wang, M., Liang, C., … Zhu, X. (2013). Effects of understory removal and nitrogen fertilization on soil microbial communities in Eucalyptus plantations. *Forest Ecology and Management*, *310*, 80-86. doi: 10.1016/j.foreco.2013.08.013

Zhao, Q., Classen, A. T., Wang, W. W., Zhao, X. R., Mao, B., & Zeng, D. H. (2017). Asymmetric effects of litter removal and litter addition on the structure and function of soil microbial communities in a managed pine forest. *Plant and Soil*, *414*(1-2), 81-93. doi: 10.1007/s11104-016-3115-7

Zhou, L., Cai, L., He, Z., Wang, R., Wu, P., & Ma, X. (2016). Thinning increases understory diversity and biomass, and improves soil properties without decreasing growth of Chinese fir in southern China. *Environmental Science and Pollution Research*, *23*(23), 24135-24150. doi: 10.1007/s11356-016-7624-y

Zimmer, M. (1997). Surfactants in the gut fluids of Porcellio scaber (Isopoda: Oniscidea), and their interactions with phenolics. *Journal of Insect Physiology*, *43*(11), 1009-1014. doi: 10.1016/S0022-1910(97)00074-7

Zimmer, M., Kautz, G., & Topp, W. (2005). Do woodlice and earthworms interact synergistically in leaf litter decomposition? *Functional Ecology*, *19*(1), 7-16. doi: 10.1111/j.0269-8463.2005.00926.x

Zou, C., Sands, R., Buchan, G., & Hudson, I. (2000). Least limiting water range: A potential indicator of physical quality of forest soils. *Australian Journal of Soil Research*, *38*(5), 947-958. doi: 10.1071/SR99108

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## Scientific publications

Peng, Y., Yang, W., Li, J., **Wang, B.**, Zhang, C., Yue, K., Wu, F. (2015). Contribution of soil fauna to foliar litter-mass loss in winter in an ecotone between dry valley and montane forest in the upper reaches of the Minjiang River. *Plos one*, 10(4), e0124605.

Peng, Y., Yang, W. Q., **Wang, B.**, Zhang, H. L., Yue, K., Wu, F. Z. (2015). Heavy metal output and content of headwater streams in an alpine forest in the upper reaches of the Yangtze River. *Fresenius Environmental Bulletin*, 24(1), 132-138.

**Wang, B.**, Wu, F., Xiao, S., Yang, W., Justine, M. F., He, J., Tan, B. (2016). Effect of succession gaps on the understory water-holding capacity in an over-mature alpine forest at the upper reaches of the Yangtze River. *Hydrological Processes*, 30(5), 692-703.

Liao, S., Ni, X., Yang, W., Li, H., **Wang, B.**, Fu, C., Xu, Z., Tan, B., Wu, F. (2016). Water, rather than temperature, dominantly impacts how soil fauna affect dissolved carbon and nitrogen release from fresh litter during early litter decomposition. *Forests*, 7(10), 249.

Ni, X., Yang, W., Qi, Z., Liao, S., Xu, Z., Tan, B., **Wang, B.**, Wu, Q., Fu, C., You, C., Wu, F. (2017). Simple additive simulation overestimates real influence: altered nitrogen and rainfall modulate the effect of warming on soil carbon fluxes. *Global change biology*, 23(8), 3371-3381.

Landuyt, D., De Lombaerde, E., Perring, M. P., Hertzog, L. R., Ampoorter, E., Maes, S. L., De Frenne, P., Ma, S., Proesmans, W., Blondeel, H., Sercu, B. K., **Wang, B.**, Wasof, S., Verheyen, K. (2019). The functional role of temperate forest understorey vegetation in a changing world. *Global Change Biology*, 25(11), 3625-3641.

**Wang, B.**, Blondeel, H., Baeten, L., Djukic, I., De Lombaerde, E., Verheyen, K. (2019). Direct and understorey-mediated indirect effects of human-induced environmental changes on litter decomposition in temperate forest. *Soil Biology and Biochemistry*, 138, 107579.

**Wang, B.**, Verheyen, K., Baeten, L., De Smedt, P. (2020). Herb litter mediates tree litter decomposition and soil fauna composition. *Soil Biology and Biochemistry*, 108063.

De Lombaerde, E., Blondeel, H., Baeten, L., Landuyt, D., Perring, M. P., Depauw, L., Maes, S. L., **Wang, B.**, Verheyen, K. (2020). Light, temperature and understorey cover predominantly affect early life stages of tree seedlings in a multifactorial mesocosm experiment. *Forest Ecology and Management*, 461, 117907.

**Wang, B.**, Verheyen, K., Baeten, L., De Smedt, P. (2020). Herbaceous litter determines macro-detritivores performance and tree litter decomposition in a lab experiment. *Pedobiologia*, 2020. (Under review).

**Wang, B.**, Verheyen, K., De Smedt, P., Baeten, L. (2020). The secondary regulating effect of the herb layer on the soil surface microclimate and its effects on litter decomposition using standard tea bags. *Uunpublished*.