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

Authors: Bin Wang [a*], Haben Blondeel [a], Lander Baeten [a], Ika Djukic [b], Emiel De Lombaerde [a], Kris Verheyen [a]

[a] Forest & Nature Lab, Department of Environment, Ghent University, Geraardsbergsesteenweg 267, 9090 Gontrode, Belgium;

[b] Swiss Federal Institute for Forest, Snow and Landscape ResearchWSL, Zürcherstrasse 111, 8903 Birmensdorf, Zürich, Switzerland.

Corresponding author: bin.wang@ugent.be

Abstract

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.

1. Introduction

Litter decomposition is the dominant process of the carbon and nutrient cycles in forest ecosystems, which contributes to approximately 60 Pg 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 (Liu, 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 (Freeman, 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 and 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 and enzyme activity (DeForest, et al., 2004; Treseder, 2008; 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 (Beaten, et al., 2010; Verheyen, et al., 2012; Perring, et al., 2017). 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 (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 and Bleken, 2015). It is widely accepted that recalcitrant litter is less sensitive to environmental changes compared to labile litter. For example, the direct effects of warming and nitrogen enrichment on recalcitrant litter decomposition are considerably weaker than on labile litter, especially in the early stage of litter decomposition where most of the water-soluble substrates are released (De Long, et al., 2016; Christiansen, et al., 2017). 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 (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 land-use legacy treatments will have positive effects on the decomposition of both types of litter, whereas nitrogen enrichment will limit tea decomposition (especially of the labile litter type). (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. (iv)

The treatment effects on litter decomposition are stronger in nutrient-rich soil compared with nutrient-poor soil.

2. Material and methods

2.1. Site description

This study was conducted in the Aelmoeseneie forest (50^{o} 58.5´ N, 3^{o} 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).

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., 2018a). 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, 2018a). 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, $\mu g·kg^{-1}$) were determined as described by Blondeel et al. (2018a). Based on soil texture and bedrock properties (Table 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. (2018a) for more information.

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 A1, A2 & A3; Fig. A1 & A2). See Appendix A 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 *Fagus sylvatica*, *Quercus robur*, *Acer pseudoplatanus*, *Fraxinus 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. 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, 2018b). 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 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 and 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 B1).

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. 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.

2.5. Statistical analyses

All statistical analyses were performed using R (R Core Team 2017), and graphs were made with the *ggplot2* package (Wickham 2009). 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 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.

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. 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, ~~2004~~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.

3. Results

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 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 2; Fig. C1). 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). 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 ($P < 0.05$).

The environmental treatments (temperature, light, nitrogen, and land use) generally had a limited effect on mass loss for both tea types across soils (Table 2, Fig. 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 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 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).

3.2. 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 2). Soil type had a significantly positive effect on plant cover ($P < 0.001$, Table 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 2; Fig. 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.

3.3. Direct and indirect effects of treatments and understorey vegetation cover on tea decomposition

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. 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 lager part of indirect effect across tea types, soil fertility groups and incubation times (Fig. 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.

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.

4.1 The 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 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. 2). On one hand, 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 become water-stable soil aggregates (Angst, et al., 2017). On the other hand, 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. C1).

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.

4.2 Direct and indirect effects of environmental changes on litter decomposition

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. Due to the changes of rainfall, light interception, and water evapotranspiration, increased plant cover is expected to slow the rate of increasing in soil temperature, which may also influence the water balance in the soil (Wahren et al., 2005; Niinemets, 2010; Myers-Smith et al., 2011; 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; Li 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. 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. 5). The higher concentrations of phosphorus, which generally promotes microbial degradation processes when systems are less N-limited, 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. 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, and 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. 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. 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

BW was supported by the China Scholarship Council while studying in the Ghent University (No. 201606910080). This research was funded by the European Research Council through a Consolidator Grant (grant no. 614839: PASTFORWARD) attributed to KV. We are thankful to UNILEVER for sponsoring the Lipton tea bags and to ILTER initiative grant supporting the work within the TeaComposition initiative. Moreover, we appreciate the Laboratory of Plant Ecology (represented by Kathy Steppe), Faculty of Bioscience Engineering, Ghent University, who kindly supported us temperature and precipitation data in Aelmoeseneie forest. We are greatly thankful to the people who assisted with field and laboratory work associated with this research, including Adrien Berquer, Dr. Lionel Hertzog, Shiyu Ma and Çağla Elif Garip.

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Figure captions

Figure 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.

Figure 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. 5.

Figure 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).

Figure 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.

Figure 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 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. 5, the indirect effects are calculated as a product of the direct effect of treatments on plant cover and direct effects of plant cover on tea decomposition. The omitted columns had represented insignificant pathways ($p < 0.05$).

Tables

Table 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±58.90 ^{a}	380.00±68.10 ^{a}	468.00±117.90 ^{a}	298.00±61.60 ^{B}	602.00±72.20 ^{A}	694.00±91.30 ^{A}
Ca (mg·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$_2$O)	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).

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