Soil heterogeneity in tree mixtures depends on spatial clustering of tree species

Bram K. Sercu\textsuperscript{a,\ast,\dagger}, Lionel R. Hertzog\textsuperscript{a,1}, Stefanie R.E. De Groote\textsuperscript{b}, Lander Baeten\textsuperscript{b}, Luc Lens\textsuperscript{a}, An Martel\textsuperscript{c}, Dries Bonte\textsuperscript{a}, Kris Verheyen\textsuperscript{b}

\textsuperscript{a}Department of Biology, Terrestrial Ecology Unit (TEREC), Ghent University, K.L. Ledeganckstraat 35, 9000 Gent, Belgium
\textsuperscript{b}Department of Environment, Forest & Nature Lab, Ghent University, Geraardsbergsesteenweg 267, 9090 Gontrode, Belgium
\textsuperscript{c}Department Pathology, Bacteriology and Avian Diseases, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

Received 14 August 2018; accepted 30 June 2019
Available online 9 July 2019

Abstract

Heterogeneity in soil characteristics promotes and maintains coexistence between a diverse set of species. In forests, trees have species-specific impacts on soil abiotic characteristics and mixing of tree species is being promoted as a tool to ensure high levels of diversity and functioning. Yet, limited knowledge is available on the effect of tree species composition and spatial clustering on heterogeneity in soil characteristics. In this paper we derived heterogeneity of key characteristics of the leaf litterfall, the forest floor and the mineral topsoil (C, N and base cation concentration, C:N ratio and mass) in 53 plots of 7 different tree species compositions. We found that heterogeneity increased from the leaf litterfall, through the forest floor down to the mineral topsoil. Mixing tree species did not lead to an increased heterogeneity in the forest floor and topsoil compared to monocultures. However, we did find that mixed plots where conspecific trees stand in groups are more heterogeneous than plots where species are intimately mixed. Our results imply that heterogeneity in soil characteristics does not necessarily increase with tree diversity, but that within mixed stands the spatial organization of tree species should be considered in relation to the scale at which heterogeneity is desired.

© 2019 Gesellschaft für Ökologie. Published by Elsevier GmbH. All rights reserved.

Keywords: Species diversity; Fagus sylvatica; Forestry; Forest floor; Leaf litter; Quercus robur; Quercus rubra

Introduction

Heterogeneity in resource supply is key to the dynamics, interactions and coexistence of species (Chesson 2000; Chase & Leibold 2003). Spatial and temporal variation in resources should promote coexistence between a diverse set of organisms by increasing available niche resource space (Silvertown 2004; Nielsen et al. 2010; Baldrian 2017). At the same time, species also impact environmental conditions through their functional traits (Hooper et al. 2005), so that functionally diverse communities may create and maintain
resource heterogeneity (Cadotte, Carscadden, & Mirotchnick 2011).

Forests are a good model system to study the role of biodiversity in creating spatial resource heterogeneity, since trees generally have strong and species-specific effects on their neighboring environment (Jones, Lawton, & Shachak 1997). When trees shed leaves in autumn, the leaf litterfall layer is formed which gradually decomposes into the forest floor which eventually leaches nutrients to the topsoil. Each of these compartments provides a habitat for many species groups. Several studies have compared monocultures of different tree species and showed that they differ in litter, forest floor and mineral soil properties (Vesterdal, Schmidt, Callesen, Nilsson, & Gundersen 2008). These differences in turn determine the composition, diversity and abundance of other biotic groups such as the soil microbial community (Baldrian 2017), earthworms (Schellhout et al. 2017), the oribatid mite community (Wardle, Yeates, Barker, & Bonner 2006) and the herb community (Augusto, Dupouey, & Ranger 2003). As a result, it is often explicitly or implicitly assumed in the literature that mixed forest stands will have a higher niche diversity and spatial resource heterogeneity compared with monocultures, so that they can harbor a higher diversity of soil biota and herb species (Reich, Frelich, Voldseth, Bakken, & Adair 2012; Ampoorter et al. 2016).

This assumption relies on the spatially limited and species-specific effects of tree individuals on soil characteristics. This can be conceptualized as a belowground zone of influence of individual trees (May, Grimm, & Jettsch 2009). However, there is little to no information on the spatial extent of the influence of an individual tree in determining soil properties. In a simplified scenario, each point of the forest soil is influenced purely by one species (Fig. 1A). Some studies find that spatial patterns of some soil properties with the canopy projection of tree species converges (Lechowicz & Bell 1991; Rodriguez, Durán, Fernández-Palacios, & Gallardo 2009). However, other litter, forest floor and topsoil variables in these studies show spatial patterns that seem independent of the spatial position of trees. Differences in nutrient stocks and ratios in the forest floor and topsoil are mainly driven by leaf litterfall (Augusto, Ranger, Binkley, & Rothe 2002). The spatial positioning of the trees, the prevailing wind direction, wind speed, and the species leaf shedding attributes, all might lead to spatial patterns of leaf litterfall in mixtures that differ from the spatial pattern of tree crowns (Jonard, Andre, & Ponette 2006).

At a small spatial scale (i.e., stand level), the nature of the spatial mixing of tree species (intimate mixtures vs. clustered groups) could have consequences for variability in litter, forest floor and topsoil characteristics. Stands where conspecific trees are clustered in small groups could lead to high stand-level heterogeneity with distinct zones of monoculture influence and a mixed zone (Fig. 1C). On the other hand, intimate mixing of tree species could lead to lower heterogeneity via the creation of homogeneous conditions that are intermediate between monocultures as is illustrated in Fig. 1B (Thomsen, Svenning, & Balslev 2005; Ampoorter et al. 2016). This could explain why most studies investigating the relationship between overstory and understory diversity found no evidence for a positive relationship between tree and understory diversity (Thomsen et al. 2005; Ampoorter et al. 2016). Therefore, the implicit assumption that mixing tree species increases environmental heterogeneity needs empirical testing as the effect of intimate mixing on variation may be more complex than commonly expected.

Here we studied the effect of tree species composition and spatial clustering on the spatial heterogeneity of mass and nutrient concentration in the litter, forest floor and top soil in order to test whether spatial heterogeneity increases in tree mixtures relative to monocultures. The study was performed in a tree diversity research platform (TREEWEB), where we sampled three tree species and all their potential combinations: (i) beech (Fagus sylvatica), (ii) pedunculate oak (Quercus robur) and (iii) red oak (Quercus rubra). These three species have different leaf nutrient concentration (De Groote et al. 2017) but all belong to the low-end of the leaf quality spectrum (Vesterdal et al. 2008). In this paper, we ask the following questions: what is the level of spatial nutrient heterogeneity in the leaf litter, forest floor and mineral topsoil? Is within-plot spatial nutrient heterogeneity of leaf litter, forest floor and topsoil higher in tree mixtures compared with monocultures? What is the expected spatial nutrient heterogeneity in mixtures based on monoculture information? What is the effect of tree clustering on within-plot spatial nutrient heterogeneity?

Materials and methods

Study area

Data were collected across 53 plots (30 m × 30 m) located in 19 forest fragments (fragment size ranged from 1.31 to 90.36 ha with an average of 28.13 ha) in northern Belgium (the “TREEWEB” platform; Appendix A: Fig. 3 in Supplementary material; De Groote et al. 2017). The climate is temperate and characterized by a mean annual temperature of 9.5 °C and an annual precipitation of 726 mm evenly distributed over the year (1980–2010, Royal Meteorological Institute of Belgium). As land use legacy effects can influence soil conditions in forests (e.g. high phosphorus content in post-agricultural soil), all plots were selected in forest stands that had been continuously forested for at least the last 150 years. The plots were selected to vary principally in tree species composition, while minimizing the variation in other environmental variables such as soil texture. Plots had dry, sandy loam soil and were located in mature, extensively managed forest stands, which showed no signs of management for the last 10–15 years. The tree species pool was formed of three regionally common tree species (Q. robur, Q. rubra, and F. sylvatica). Q. rubra is locally invasive and thus of concern to both forest managers and policy makers. Each of
the seven possible species combinations (1. Q. robur; 2. Q. rubra; 3. F. sylvatica; 4. Q. robur–Q. rubra; 5. Q. robur–F. sylvatica; 6. Q. rubra–F. sylvatica; 7. Q. robur–Q. rubra–F. sylvatica) was included in the design, with six to eight replicates for each combination. The exact location of the stems was mapped and total basal area of the tree stems was calculated using a Field-Map system (www.field-map.com). Plots were selected so that the proportion of non-target tree species was minimized (<5% of the basal area) and the evenness of the target tree species in mixtures was maximized (>60% of maximum Shannon evenness based on basal area) (Baeten et al., 2013). The study plots had a mean stem number of 16 trees per plot (stem density: 178 trees/ha; range: 100–333 trees/ha, standard error: 7.85 trees/ha) and a mean basal area of 38.58 m²/ha (range: 25.09–52.48 m²/ha, standard error: 0.85 m²/ha). Each plot was subdivided into four 15 m × 15 m squares. Five subplots of 5 m × 5 m were established, one in the center of each square and one in the center of the plot, that were used for standardized collection of litterfall, forest floor and topsoil samples (Appendix A: Fig. 4 in Supplementary material).

Litterfall, forest floor and topsoil sample collection

Full details on the data collection are given in De Groote et al. (2018), the most important points are outlined below. We refer the interested reader to the above-mentioned publication for further information. As we aimed to generate generalizations across gradients of tree diversity, rather than to document detailed fine-scale patterns in few locations, we derived the spatial heterogeneity from a restricted number of samples per plot (N = 3), but maximized replication of these local heterogeneity indices across forest plots (N = 53).

Leaf litterfall

Leaf litterfall was sampled via 1 m tall litterfall traps with a surface area of 0.24 m². Litterfall traps were placed in three of the five subplots in all plots: one was placed in the center of the plot, one northeast of the center and one either northwest or southeast of the center. Litterfall was collected from all 159 traps every two weeks from September 2014 until January 2015, resulting in eight collection dates. Litterfall samples were pooled later on in the processing (see ‘derived variables’) to obtain one value per litterfall trap. The litterfall samples were oven-dried at 65 °C. Litterfall material was weighed to the nearest 0.01 g and samples for chemical analysis were ground, keeping the leaves of each target tree species separately. Leaf litterfall from non-target species (i.e. shrub species such as Corylus avellana) was pooled together and was ignored in the analysis, if they accounted for less than 5% of the total litter biomass (in 27 out of 53 plots; see Table 2 in De Groote et al. 2018). Heterogeneity levels in the 27 plots where non-target species litter was ignored did not differ from the other 26 plots (Appendix A: Fig. 5 in Supplementary material).

Forest floor

The forest floor was sampled once using 25 cm × 25 cm wooden frames at the same three subplots as the litterfall traps in March 2016. The recently fallen rather intact leaves were removed and the fragmentation and humification layer, containing the fragmented and (partly) decomposed leaves was processed further. Forest floor samples were oven-dried at 65 °C twigs, fruits and non-litter material (e.g. moss) were removed and the remaining fraction was weighed to the nearest 0.01 g. Samples for chemical analysis were ground.

Topsoil

The mineral soil was sampled once using a soil core (diameter: 3 cm) at three random locations per subplot in the same three subplots used for leaf litterfall and forest floor sampling between August and September 2014. The three replicates per subplot were pooled in subsequent processing. Soil sampling was done at the same subplots of the litterfall and forest floor.
Table 1. Average and standard deviation values (in brackets) of all modelled variables (Mass, C: carbon concentration, N: nitrogen concentration, CN: carbon:nitrogen ratio, BC: base cation concentration) for the different tree species composition (Fsyl: Fagus sylvatica, Qrob: Quercus robur, Qrub: Quercus rubra) in the leaf litterfall, forest floor and topsoil.

<table>
<thead>
<tr>
<th>Horizon</th>
<th>Variable</th>
<th>Fsyl</th>
<th>Qrob</th>
<th>Qrub</th>
<th>FsylQrob</th>
<th>FsylQrub</th>
<th>QrobQrub</th>
<th>FsylQrobQrub</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf litter</td>
<td>Mass (kg/m²)</td>
<td>88.43 (11.44)</td>
<td>112.3 (12.22)</td>
<td>99.19 (17.92)</td>
<td>99.05 (11.36)</td>
<td>104.46 (11.71)</td>
<td>105.43 (15.53)</td>
<td>107.34 (11.16)</td>
</tr>
<tr>
<td></td>
<td>C (%)</td>
<td>49.96 (1.55)</td>
<td>51.11 (1.31)</td>
<td>52.17 (1.73)</td>
<td>49.47 (1.34)</td>
<td>51 (1.26)</td>
<td>50.95 (2.01)</td>
<td>50.17 (1.61)</td>
</tr>
<tr>
<td></td>
<td>N (%)</td>
<td>1.14 (0.10)</td>
<td>1.39 (0.13)</td>
<td>0.94 (0.16)</td>
<td>1.19 (0.07)</td>
<td>1.09 (0.09)</td>
<td>1.21 (0.12)</td>
<td>1.10 (0.10)</td>
</tr>
<tr>
<td></td>
<td>CN</td>
<td>43.79 (2.74)</td>
<td>37.22 (3.66)</td>
<td>57.31 (7.51)</td>
<td>41.43 (2.83)</td>
<td>48.05 (4.50)</td>
<td>44.3 (5.08)</td>
<td>47.19 (5.19)</td>
</tr>
<tr>
<td></td>
<td>BC (cmol/kg)</td>
<td>563.67 (80.91)</td>
<td>730.53 (92.19)</td>
<td>589.11 (60.01)</td>
<td>703.32 (91.98)</td>
<td>525.98 (58.05)</td>
<td>632.29 (81.85)</td>
<td>643.62 (161.75)</td>
</tr>
<tr>
<td>Forest floor</td>
<td>Mass (kg/m²)</td>
<td>47.24 (26.31)</td>
<td>36.94 (24.47)</td>
<td>24.18 (21.22)</td>
<td>35.88 (21.49)</td>
<td>32.73 (15.07)</td>
<td>22.04 (17.52)</td>
<td>38.99 (24.37)</td>
</tr>
<tr>
<td></td>
<td>C (%)</td>
<td>46.73 (3.82)</td>
<td>42.38 (5.55)</td>
<td>44.15 (8.65)</td>
<td>43.81 (5.86)</td>
<td>44.54 (8.44)</td>
<td>42.35 (5.77)</td>
<td>45.58 (5.22)</td>
</tr>
<tr>
<td></td>
<td>N (%)</td>
<td>2.05 (0.18)</td>
<td>2.10 (0.23)</td>
<td>1.77 (0.39)</td>
<td>2.00 (0.23)</td>
<td>1.94 (0.42)</td>
<td>1.88 (0.25)</td>
<td>2.01 (0.23)</td>
</tr>
<tr>
<td></td>
<td>CN</td>
<td>22.80 (1.50)</td>
<td>20.16 (1.26)</td>
<td>25.25 (3.24)</td>
<td>21.93 (1.27)</td>
<td>23.10 (1.66)</td>
<td>22.53 (1.98)</td>
<td>22.75 (1.35)</td>
</tr>
<tr>
<td></td>
<td>BC (cmol/kg)</td>
<td>315.4 (100.22)</td>
<td>395.4 (95.25)</td>
<td>368.44 (118.46)</td>
<td>396.5 (98.44)</td>
<td>301.56 (81.96)</td>
<td>391.52 (80.87)</td>
<td>319.69 (88.87)</td>
</tr>
<tr>
<td>Topsoil</td>
<td>Mass (kg/m²)</td>
<td>0.19 (0.04)</td>
<td>0.27 (0.11)</td>
<td>0.18 (0.06)</td>
<td>0.30 (0.14)</td>
<td>0.21 (0.08)</td>
<td>0.30 (0.07)</td>
<td>0.24 (0.06)</td>
</tr>
<tr>
<td></td>
<td>N (%)</td>
<td>16.10 (2.06)</td>
<td>15.19 (1.40)</td>
<td>15.41 (1.83)</td>
<td>16.93 (1.25)</td>
<td>17.55 (2.41)</td>
<td>16.07 (1.32)</td>
<td>15.95 (1.94)</td>
</tr>
<tr>
<td></td>
<td>CN</td>
<td>5.98 (3.31)</td>
<td>15.83 (8.06)</td>
<td>4.84 (2.86)</td>
<td>18.56 (17.88)</td>
<td>6.42 (3.66)</td>
<td>12.61 (5.44)</td>
<td>10.31 (5.46)</td>
</tr>
</tbody>
</table>
sampling and was confined to the upper 10 cm of soil. All soil samples were dried at 40 °C to constant weight and samples for chemical analysis were crushed and sieved through a 1 mm mesh.

**Chemical analysis**

Leaf litterfall chemical characteristics were analyzed per species and per plot pooling together the leaf materials over the 8 collecting dates and over the three subplots.

Total nitrogen and carbon concentration was measured by dry combustion using an elemental analyser (Vario MAX CNS, Elementar, Germany). Exchangeable potassium, calcium and magnesium concentration was measured by atomic absorption spectrophotometry (AA240FS, Fast Sequential AAS) after extraction in BaCl2 (NEN 5738:1996).

**Derived variables**

Three types of response variables were derived: (i) mass, (ii) elemental concentration and (iii) stoichiometric ratios. We used leaf litterfall mass and forest floor mass. For each leaf litterfall trap, the dried leaf mass was summed across the temporal replicates. For the forest floor, the sum of the dried fragmentation and humification layer was computed for each subplot. The concentration variables used were the carbon, nitrogen and base cations concentration. These were derived per subplot for the litterfall, forest floor and topsoil. The base cation concentration were calculated by summing the atomic equivalents (mmole/kg) of potassium, calcium and magnesium taking into account the valence and the atomic mass of the elements. Finally the C:N ratio was calculated for the litterfall, forest floor and topsoil in each subplot. In the leaf litterfall, the concentrations were derived per species and per plot. We calculated the values per leaf litterfall trap as the weighted average of the concentration per tree species, with the weights being the dry mass of leaf litterfall from the tree species from each trap.

As an additional explanatory variable, a clustering index was derived for each plot based on the locations of the tree stems from the Field-Map analysis. This index represents the proportion of trees in the plot that have a conspecific as the nearest tree neighbor. Low values indicate that most trees in the plot have another species as nearest neighbor and form an intimate mixture. High values mean that most trees have a conspecific as the nearest neighbor, which indicates that species are clustered.

**Data analysis**

A full description of the data analysis is given in Appendix A: Extended Analysis section in Supplementary material; we present here only the core information. For each response variable, (i) mass (ii) carbon concentration, (iii) nitrogen concentration, (iv) C:N ratio and (v) base cation concentration within the (a) leaf litter, (b) forest floor and (c) topsoil, we built a separate model (14 in total as mass was not relevant for topsoil). These models were Gaussian with two parameters: the mean and the residual deviation. The mean was regressed against tree basal area and species composition with a random intercept per plot. The residual deviation was modelled with a log link and was regressed against species composition with a random intercept per plot. For the purpose of this paper we present within-plot variability or heterogeneity using the coefficient of variation (CV). The CVs were calculated for each tree species composition as the estimated residual deviation divided by the estimated mean. We chose to rely on model-estimated CVs due to the limited spatial replication (3 subplots per plot) in the dataset, which prevented us from directly computing within-plot heterogeneity from the observed values. This partial-pooling approach (Gelman et al. 2013) capitalizes on the relatively large number of sampled plots to provide more reliable estimates of average within-plot heterogeneity by shrinking extreme within-plot values towards the mean. We compared those model-derived CV values for two-species and three-species mixtures with expected values based on a simplified or null scenario, which assumed a monospecific impact and only additive effects in mixtures (see Fig. 1A). Under these assumptions the calculation of the expected CV values in the mixtures can be estimated from the statistical properties of the monoculture distributions. Basically, these expectations were obtained by dividing the average of the estimated standard deviations by the average of the estimated means from monocultures of the tree species present in the mixtures. More details and caveats of this approach are given in Appendix A in Supplementary material.

In a second step, we added the plot-level clustering value as a covariate in the regression on the residual variation to explore the effect of clustering vs. intimate mixing on the CVs. The dataset used for this analysis included only the species combinations with two or three species.

All models were fitted with a Bayesian approach, using the Stan probabilistic language through R v3.3 (R Core Team 2016) with the package ‘brms’ v1.10 (Bürkner 2016).
Results

Observed mean and variation of the explored variables in the leaf litterfall, forest floor and topsoil and for the nutrient and mass variables are shown in Table 1. The overall within-plot variation (quantified as the coefficient of variation CV) generally increased from the leaf litterfall to the topsoil (Fig. 2). This pattern was less marked for the C:N ratio than for the other variables. The highest CV was observed for the mass of the forest floor which was considerably higher (median 0.5) than CV of leaf litterfall mass (median 0.09).

Tree composition effect on heterogeneity

When comparing monoculture plots, we found that in the leaf litterfall, Q. rubra had a higher CV of litterfall mass, nitrogen concentration and C:N ratio than the other monocultures (Fig. 3). This pattern was also in part present in the forest floor where Q. rubra had a higher CV of nitrogen concentration and C:N ratio than the other monocultures. In the topsoil Q. robur had a higher CV of carbon and nitrogen concentration than the other monocultures.
In the mixture plots, for the leaf litterfall the mixture of *Q. robur*–*Q. rubra* tended to have higher CV of nitrogen concentration and C:N ratio than other 2-species mixtures. Interestingly, litterfall mass did not have a higher CV in 2 and 3-species mixtures compared to monocultures. Few consistent patterns emerged from the forest floor heterogeneity for the mixtures, but 3-species mixtures tended to have lower CV than 2-species mixtures. In the topsoil, the only marking feature was the very low CV for C:N ratio for *Q. robur–Q. rubra* mixtures. While all other species combinations had CVs around 0.08, *Q. robur–Q. rubra* mixtures had a CV of 0.05.

**Observed vs expected variation in the mixtures**

A general pattern found across tree species mixtures for mass and nutrients in the litterfall, forest floor and topsoil, was that the observed CV was lower than expected based on the monocultures (Fig. 3). In other words, expecting that the heterogeneity of a mixture is based on an additive combination of monoculture effect of the individual species (Fig. 1A) led to a general overestimation of the observed heterogeneity. Some deviation from this general pattern emerged for instance in the forest floor where the CV of carbon and nitrogen was identical or even larger than expected in *F. sylvatica–Q. rubra* and *F. sylvatica–Q. robur* mixtures. In addition, the CV of forest floor mass in *F. sylvatica–Q. robur* and *Q. robur–Q. rubra* mixtures was similar to expected values. This pattern was also found in the topsoil where mixtures of *F. sylvatica–Q. rubra* and *F. sylvatica–Q. robur* had similar observed and expected CV for carbon and nitrogen. Overall, *F. sylvatica–Q. rubra* and *F. sylvatica–Q. robur* mixtures led to a high CVs, which were closer to the expected CV.

**Nearest neighbor effect**

The spatial clustering of tree species in the mixtures had an effect on soil heterogeneity. Generally, high species clustering levels led to higher CV of nutrients in litterfall, forest floor and topsoil and mass of the forest floor (Fig. 4). Only one variable departed from this pattern: litterfall mass CV was highest in plots where tree species were intimate mixtures (i.e. low clustering).

**Discussion**

Four general patterns emerged from our results: (i) heterogeneity increased from leaf litterfall to topsoil, (ii) within-plot heterogeneity in forest floor and topsoil characteristics did not increase in tree mixtures compared to monocultures, (iii) the observed heterogeneity in mixtures was generally lower than expected based on a simple scenario and (iv) spatial clustering of tree species affected within-plot heterogeneity: in the litterfall clustering reduced heterogeneity, while in the forest floor and topsoil clustering increased heterogeneity.

**Increase in heterogeneity towards deeper strata**

Leaf litterfall mass and chemical composition was relatively homogeneous throughout the plots, even in mixtures. This may be due to the similarity in litterfall properties of the three species: they are broad-leaved tree species with relatively similar and low leaf litterfall quality (*Vesterald et al. 2008*). Despite the homogeneous leaf litterfall, the forest floor and topsoil showed a high heterogeneity. Forest floor mass was the variable showing the highest heterogeneity but also concentration of the nutrients were more variable in the forest floor and topsoil compared to the leaf litter. We need to be cautious with the comparison of variability in litterfall, forest floor and topsoil since the sampling area and protocol differed between the layers. Specifically, the sampling area was highest for the leaf litterfall (0.24 m²), lower for the forest floor (0.0625 m²) and lowest for the topsoil (0.0027 m²). Nevertheless, we believe that the large variability in the topsoil and forest floor clearly indicates that other processes than tree litterfall composition drive variation in the forest floor and topsoil.

Other studies also found high stand-level spatial heterogeneity of soil nutrients and microbial biomass in temperate forests (*Gömöryová 2004; Baldrian, Merhautová, Cajthaml, Petránková, & Šnajdr 2010*). Climatic variables such as humidity, light and temperature are major drivers of decomposition rates on broad geographical scales (*Bradford, Berg, Maynard, Wieder, & Wood 2016*), but they can also differ on a scale of meters and create different microclimates below the canopy. For instance, due to the structure and position of canopy, seasonal precipitation can be twice as high only few meters apart (*Staelens, De Schrijver, Verheyen, & Verhoest 2006*). These microclimatic differences might create consistent differences in decomposer activity and decomposition rates (*Shaw & Harte 2001, but see Köchy & Wilson 1997*), which in turns leads to high heterogeneity in the mass and nutrient concentration of the forest floor. Subsequently, organic matter and nutrients are gradually incorporated into the topsoil horizon via bioturbation and leaching. Since differences accumulate over time, spatial heterogeneity of the nutrients in the topsoil horizon increases even further (*Trum, Titeux, Ranger, & Delvaux 2011*).
scenario, Fig. 1A) but was a more homogeneous mix of litter of the different tree species present in the plot (Fig. 1B); or 2) that phenotypic plasticity in leaf nutrient concentration led to lower variation in the mixtures than in the monocultures (Pérez-Suárez, Arredondo-Moreno, Huber-Sannwald, & Vargas-Hernández 2009). During leaf shedding, although most leaves end up close to the tree of origin, part of the leaves are carried up to 40 m away from the tree (Jonard et al. 2006; Rothe & Binkley 2001). The litterfall thus is a mix of leaves of several of the surrounding trees. In mixed plots, the relative influence of each species varies spatially, some locations have a litterfall dominated by one species while other locations have equal proportions of leaves from different species. Although all three tree species in our study have poor leaf quality (Vesterdal et al. 2008), they are significantly different from each other (see Table 1 and De Groote et al. 2017). Leaf litterfall quality is a driving force in determining decomposition rate (Bradford et al. 2016) and other studies found that our three study species differ significantly in decomposition rate and weight loss (Hobbie et al. 2006; Jonard, Andre, & Ponette 2008). From the spatial variation in leaf litterfall quality in mixtures and the differences in decomposition rate, we can expect a higher heterogeneity of mass and nutrient concentration of the forest floor and topsoil in mixtures compared to monocultures. However, both in the forest floor and topsoil horizons, the heterogeneity of mass and nutrient concentration in mixtures was equal to monoculture values or only slightly higher. Similar to litterfall, the observed heterogeneity in the forest floor and topsoil was generally lower than the expectations from the simplified scenario which assumes that each point of the forest soil was influenced purely by one species. The similar levels of heterogeneity in mixtures and monocultures may indicate: that litterfall quality is not a driving force in decomposition in our system, that non-additive effects are present (Gartner & Cardon 2004; Lummer, Scheu, & Butenschoen 2012), or that the studied tree species had too similar litter properties to establish strong patterns (Vesterdal et al. 2008). Micro-environmental differences might be more important than chemical quality of the leaves in the creation of heterogeneous conditions in the forest floor and topsoil for our study system. Moreover, belowground factors such as root distribution or root functional traits have also been shown to mediate the effect of tree species on topsoil nutrient distribution via changes in root chemistry such as C/N ratios (Hishi 2007). Further studies encompassing a broader gradient of litterfall quality and accounting for belowground effects of trees would improve our knowledge on the effect of tree mixtures on heterogeneity in the forest floor and topsoil.

Tree clustering affects within-plot heterogeneity

The effect of individual trees on soil characteristics was spatially constrained. Spatial organization of tree species will therefore affect heterogeneity within a stand. We found higher within-plot heterogeneity when trees were clustered instead of intimately mixed. Most forest biodiversity and ecosystem function experiments implement a strict intimate mixing design to plots of a fixed size (Scherer-Lorenzen et al. 2005). While intimate mixtures enable interspecific interactions and crown complementarity, which is one of the main mechanism behind the positive tree diversity – productivity relationship (Williams, Paquette, Cavender-Bares, Messier, & Reich 2017), this study shows it does not lead to higher soil heterogeneity. Intimate mixing of tree species leads to homogenization in environmental conditions. This might explain why many studies do not find a positive relationship between tree diversity and understory diversity (e.g. Thomsen et al. 2005; Ampoorter et al. 2016). As a result, the relationships between biodiversity and ecosystem functions from experiments may be affected by the specific planting regime and the resulting levels of heterogeneity. Care should be taken when considering such plots as a model for processes that respond to environmental conditions at small spatial scales such as microbial or plant communities. However, it is important to note that observed heterogeneity levels depend on the spatial scale of the measurements. Studies should be carefully tailored to assess heterogeneity at the relevant scale for the process under investigation. Our results imply that tree species clustering might increase soil heterogeneity on a scale of meters but this is not necessarily true on smaller scales of decimeters, which may be relevant for microbial diversity (Saetre 1999), or on larger scale in landscapes.

Conclusion

We did not find strong evidence for the assumption that mixed forest stands will have a higher niche diversity and spatial resource heterogeneity compared with monocultures, and therefore could harbor a higher diversity of soil biota and herb species. Species-specific impacts were present in our study and more clustered spatial configurations of tree species led to higher heterogeneity than intimately mixed plots. However, in general, variances were similar across species combinations in the forest floor and topsoil which indicates that tree species characteristics are not the main drivers of heterogeneity for the studied species. In all species combinations, the forest floor and the topsoil had a high spatial heterogeneity in nutrient conditions. Further research would be needed to confirm our suggestion that micro-environmental differences that are unrelated to tree identity such as precipitation, temperature and light might be the major drivers of nutrient heterogeneity in the forest floor and topsoil via differences in decomposition.

Acknowledgements

We want to thank our collaborators in the TREEWEB project. Funding was provided by FWO-Vlaanderen via a
doctoral fellow grant (B.K.S.) and via the UGent GOA project Scaling up Functional Biodiversity Research: from Individuals to Landscapes and Back (TREEWEB). We also wish to express our gratitude to Teja Tscharntke and three anonymous reviewers for providing helpful feedback and critical comments.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.biocevo.2019.06.007.

References


