Species identity and distance to the tree affect topsoil chemistry more than mixture effects in mature forest

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Abstract

Trees effect topsoil chemistry, and the strength and direction of these effects vary depending on the tree species identity. Tree species with nutrient-poor litter have the potential to degrade soil fertility, which is characterized by high acidity, low availability of essential nutrients for plant growth and high levels of available Al and Fe. In contrast, species with more easily decomposing, nutrient-rich litter can ameliorate the soil quality. In this study, we are investigating the effects of tree species identity on topsoil chemistry on a small spatial scale. Our study site, situated in Białowieża Forest in Poland, replicates mature monocultures and two-species mixtures, using a pool of four tree species. Soil was sampled at a meter distance to the base of a tree and in the centre along a transect between two trees. We found that the total C concentration, plant available P and base cation concentration, and C:N ratio were all larger close to the tree. The pH was unaffected by the distance. We identified clear species identity effects on this distance effect. In particular, a contrast between nutrient-rich and nutrient-poor species for pH and base cations was found. However, no non-additive diversity effects in mixtures could be distinguished. Our results highlight the ameliorating effects of admixing nutrient-rich species with nutrient-poor species and the importance of tree species choice in regard to the topsoil chemical composition on a small scale.

Text

Introduction

Trees affect the biotic and abiotic characteristics of the soil. The nature of these effect depends on the "identity" of the tree species through variation in, for instance, vertical and horizontal root length (Brassard et al. 2011), acidification by root exudates (Eviner and Chapin III 2003), and interception of acidic atmospheric deposition (De Schrijver et al. 2007). Tree species notably differ in the quality and quantity of their litter, which affects its decomposition rate (Hobbie et al. 2006). Species with poor litter quality (high C:N ratio and low base cation (BC) concentration) cause litter accumulation in the forest floor, due to a slow decomposition rate (Hobbie et al. 2006). This, subsequently, leads to soil acidification accompanied by a low availability of BC and a high concentration of available Al and Fe, that are toxic to plants, soil fauna and microbiota (Sumner et al. 1991). This results in a shift in species composition of the microbial community from bacteria to fungi.
dominated (Blagodatskaya and Anderson 1998) and a reduced mixing of organic material in the soil due to a decrease in activity of soil macrofauna (Schelfhout et al. 2017), which will further slow decomposition (Muys et al. 1992). The toxic levels of Al and Fe, combined with the low availability of BC can also lead to a shift in understory composition (Verstraeten et al. 2013) and a decline in tree health and increased tree mortality (Tomlinson 2003; Hevia et al. 2019). Several studies have demonstrated the benefits of admixing species with nutrient-rich litter to improve soil chemistry, forest vitality and productivity (Carnol and Bazgir 2013; Aerts et al. 2017; Kooch and Bayranvand 2017; Desie et al. 2020). These improved soil conditions can be explained by the additive effects of incorporating faster decomposable litter with higher concentrations of BC.

In addition, when multiple tree species are planted together, we may also expect effects that differ from the additive effects of the species present, due to complementarity effects induced by interspecific interaction between species or niche partitioning (Loreau et al. 2001). One driver behind these non-additive “diversity” effects is an altered litter decomposition rate (Kou et al. 2020). Litter-diversity effects on decomposition rate can be positive due to transfer of nutrients and decomposition-stimulating compounds from nutrient-rich litter to nutrient-poor litter, which increases the decomposition rate of the nutrient-poor litter. Another process that can be distinguished is improved microclimatic conditions and greater habitat variability when litter is mixed. Negative effects have also been found and can be explained by the transfer of compounds that inhibit decomposition (Hättenschwiler et al. 2005). A more diverse soil community in diverse forest stands (Thoms et al. 2010; Korboulewsky et al. 2016) is another driver behind these diversity effects. The level of diversity in soil organisms affects the litter decomposition rate due to niche complementarity and competition (Hättenschwiler et al. 2005). Further, many studies have demonstrated higher productivity in diverse forests (Pretzsch & Forrester, 2017; Zhang et al., 2012), which can also explain tree species diversity effects on the soil chemical composition. However, in a previous study (Dhiedt et al. 2021), we found no strong diversity effects in young forest plantations on the chemical composition of the soil. It has been shown that tree species effects take time to develop (De Schrijver et al. 2012; Chen et al. 2020). Looking at the literature on mature forests, positive effects of species diversity have been identified on the C stock (Schleuß et al. 2014; Dawud et al. 2016), the pH (Guckland et al. 2009; Dawud et al. 2016), the base saturation (Guckland et al. 2009), total N (Chen et al. 2021; Liu et al. 2021), and available P (Liu et al. 2021).
The above-mentioned studies focused on stand-level patterns, e.g. comparing means across plots that differ in tree species diversity or composition. It is, however, important to understand the processes at play on individual tree or neighbourhood level as well (Forrester and Bauhus 2016), since these may paint a clearer picture of mechanisms that shape variability in soil at the spatial scales where soil microbial (Hooper et al. 2000; Eisenhauer 2016), faunal (Vanbergen et al. 2007), and plant community dynamics occur (Chesson 1986). One factor shaping this horizontal heterogeneity is the distance to the tree (Zinke 1962; Koch and Matzner 1993). Nutrient inputs from leaf and root litter, root exudates, and throughfall as well as outputs by nutrient uptake are not evenly distributed over the forest soil. The magnitude of these processes decreases with the distance to the tree. Litter fall is predominantly concentrated under the canopy (Staelens et al. 2003). Additionally, litter accumulates at the base of the tree, since it forms a physical barrier for litter redistribution by for instance wind (Facelli and Pickett 1991), although this effect may be negligible in closed forests (Beniamino et al. 1991). The fine root density, and consequently the root litter accumulation, root exudates and nutrient uptake, reduces with the distance to the stem (Olsthoorn et al. 1999). Furthermore, the throughfall deposition is more concentrated close to the stem (Beier et al. 1993) and causes a lower pH around the stem (Levia and Frost 2003). The reduction in organic matter inputs at a greater distance to the tree causes a decrease in soil carbon (Spielvogel et al. 2016). Concurrent, it reduces the binding capacity of the soil and therefore the availability of many nutrients (Jenny 1980). In addition to this distance effect, the relative position to a specific tree species will in part determine the local chemical soil composition (Holzwarth et al. 2011; Paluch and Gruba 2012; Uriarte et al. 2015). This may suggest that the negative effects of poor-quality litter might be very local in mixed forests and the same is true for the ameliorating effects of admixing species with nutrient-rich litter. In this study, we want to compare the effect of different tree species on a local scale and investigate the importance of spatial patterning of individual trees in addition to overall plot-level identity and mixing effects of tree species.

Our study site is situated in Białowieża Forest in Eastern Poland, where we selected plots of two diversity levels: monocultures and two-species mixtures. Our species pool exits, in increasing order of litter quality, of Scots pine (Pinus sylvestris L.), pedunculate oak (Quercus robur L.), silver birch (Betula pendula Roth), and European hornbeam (Carpinus betulus L.), of which the former two have nutrient-poor litter and are
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important species in European forestry (Forest Europe 2020) and the latter two have more nutrient-rich litter and are frequently suggested for admixing with the goal of restoring nutrient cycles in acidified forest soils (e.g. Brandtberg et al., 2000; Kooch & Bayranvand, 2017). We sampled the soil at a meter distance to the base of the tree and in the centre along a transect between two trees. Our questions were the following: (1) does the location in relation to the trees (close to the stem or midway between two trees) and the distance between these trees affect the chemical composition; (2) is there a species identity effect and, in particular, an ameliorating effect of admixing species with nutrient-rich litter with nutrient-poor species; and (3) can we predict the effect in mixtures based on monoculture responses, i.e. are there only additive effects as opposed to additional non-additive diversity effects? We hypothesize that the total C, Olsen P, and BC concentration are higher and the pH lower close to the tree (hypothesis 1). Next, we expect the chemical composition to be dependent on the tree species identity (hypothesis 2). Lastly, our hypothesis states that we would find non-additive effects and that the chemical composition cannot be determined based solely on the distance to the tree and monoculture responses of nearby tree species (hypothesis 3).

Materials and methods

Site description

This study was conducted in October 2019 in the Białowieża Forest, which is one of the study sites of the exploratory platform of FunDivEUROPE (Baeten et al. 2013). Białowieża is situated in Poland (52.7°N, 23.9°E; altitude between 134 and 202 m a.s.l.) and has a mean annual temperature of 6.9°C and mean annual precipitation of 627 mm (Boczoń et al. 2018). The forest is classified as hemiboreal, nemoral coniferous, and mixed broadleaved-coniferous (EEA 2007).

Within the study site, 43 plots of 30 m x 30 m were selected in mature forest stands (80-180 years old) to cover a tree species richness gradient from monocultures to five-species mixtures. Each richness level was replicated with different species compositions, composed of species from the following species pool: Norway spruce (Picea abies (L.) H. Karst.), Scots pine (Pinus sylvestris L.), silver birch (Betula pendula Roth), European hornbeam (Carpinus betulus L.), and pedunculate oak (Quercus robur L.), further referred to as spruce, pine, birch, hornbeam, and oak respectively. Plots were selected to represent mainly a richness gradient, i.e. avoiding covariation with other factors such as soil conditions and management. The soil types of the studied
plots are classified as cambisol and luvisol (IUSS Working Group WRB 2015). The soil is moderately acidic, but has a high buffering capacity. The shrub layer is dominated by hornbeam.

**Plot selection**

The original exploratory platform design included 43 plots in this study site. Only plots with monocultures and two-species mixtures were considered for the present study. We decided to exclude plots with spruce as the main species, because the population of spruce trees was decaying in the forest due to a severe outbreak of the European spruce bark beetle (*Ips typographus*) (Mikusiński et al. 2018). Studies have found significant effects of a bark beetle outbreak on the soil chemistry (e.g. Kaňa et al., 2013), which is not a factor of interest in this study.

We sampled soil in 17 plots. The following criteria were used. Monocultures are plots for which the dominant target species has a relative basal area of at least 60%, while the other species have a relative basal area of less than 30%. We have eight monoculture plots: two replicates of each of the four target species (pine, birch, hornbeam and oak). Two-species mixtures consist of two dominant target species, with each a relative basal area of at least 25% and the relative basal area of one of the dominant species is not more than double of the other dominant species. We have nine two-species mixtures: two replicates of all possible combinations of the four target species, except for the combination birch and pine (zero replicates) and the combination oak and pine (one replicate), since these combinations do not occur or occur only once in the FunDivEUROPE design of the Białowieża Forest. The relative basal area of each species in all plots can be found in Supplementary A.

**Soil sampling**

In each plot we located two transects between two target species trees, i.e. two of the same target species in monocultures and two different target species in mixtures. The transects were laid out so that no tree of another species with a considerable basal area was near to the transect. We also made sure that the transects were not too close to the edge of the plots. Note that during plot selection, we ensured that in a 10 m wide buffer zone around the plot the stand composition and soil properties did not contrast those found within the plot limits. In each transect, we laid out three subplots: one close to each of the two trees (0.5m to 1m
to the centre of the tree stem) and one in the centre. This way, each plot contained six subplots, organized along two transects (Fig 1).

**Fig 1:** Visual representation of the sampling scheme. Left: locations of the 17 plots in the Białowieża Forest, Poland. Right: the sampling design within the plot. The dots denote the trees, with a different colour for each species and a size proportionate to the diameter at breast height. The crosses denote the sampling locations. In each of the 17 plots, 2 transects were laid out between two target trees (two of the same species in monocultures and two different species in mixtures). In each transect, we sampled at three locations: close to each target tree and once midway between the two target trees.

Soil was taken in each of the subplots up to a depth of 10 cm, using a clean spade, and after removing the forest floor. After homogenizing, a subsample was separated for further analysis.

**Chemical analysis**

The soil samples were dried to constant weight at 40°C for 48h, ground, sieved over a 2 mm mesh and homogenized. To analyze the total C and N concentration, the 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). Bioavailable P which is available for plants within one growing season (Gilbert et al. 2009) by extraction in NaHCO₃ (P-Olsen; according to ISO 11263:1994(E)) and colorimetric measurement according to the malachite green procedure (Lajtha et al. 1999) (Norm: ISO 11263:1994(E)). Samples were analyzed for pH-H₂O by shaking a 1:5 ratio soil/H₂O mixture for 5 min at 300 rpm and measured with a pH
meter Orion 920A with pH electrode model Ross sure-flow 8172 BNWP, Thermo Scientific Orion, USA. Exchangeable K\textsuperscript{+}, Ca\textsuperscript{2+}, Mg\textsuperscript{2+}, and Al\textsuperscript{3+} concentrations were measured by inductively coupled plasma optical emission spectroscopy (Thermo Scientific iCAP 7400 ICP-OES). We did not use the Al data, since 44\% of the observations were under the determination limit of 100 mg kg\textsuperscript{-1}. For calculation of effective cation exchange capacity (CEC) of the soils, all extracted exchangeable cations (K\textsuperscript{+}, Ca\textsuperscript{2+}, and Mg\textsuperscript{2+} in meq/kg) were summed.

One sample in a hornbeam-pine plot showed an exceptional high concentration of Ca (> 4000 mg kg\textsuperscript{-1}, and more than four times higher than the second highest observation) and pH. Since this is likely an artifact, caused by inclusion of very localized calcareous material, we decided to exclude this sample from our analysis.

**Tree influence index**

The diameter at breast height was measured for each tree in the plots with a threshold of 7.5 cm in July 2018. For each subplot we determined the influence of each nearby tree based on its distance to the subplot and its diameter. This “tree influence index” (TII), which we based on a competition index, is calculated for every subplot \(i = 1, \ldots, 102\) and every species \(j\):  

\[
TII_{ij} = \sum_{k=1}^{n_{ij}} \frac{d_{jk}}{D_{ijk}},
\]

with \(d_{jk}\) being the diameter at breast height of tree \(k\), \(D_{ijk}\) being the distance between subplot \(i\) and tree \(k = 1, \ldots, n_{ij}\). The number of trees taken into consideration \(n_{ij}\) is determined by the vertex angle \(\alpha_{ijk}\) of the isosceles triangle with a height \(D_{ijk}\) and a base \(d_{jk}\). When \(\alpha_{ijk}\) exceeds a threshold of 3\(^\circ\), its diameter over distance ratio is added to the TII. With this threshold of 3\(^\circ\), the greatest distance between a specific subplot and a tree that is assumed to influence the chemical composition and is included in the TII is 1m at minimum, 7m on average, and 15m at maximum in the case of our subplots. A tree at these distances has to have diameter at breast height of at least 7.5cm (our threshold), 37cm, and 79cm respectively to be included. To calculate the total TII of a subplot, we sum the TII of all species:  

\[
TII_i = \sum_{j=1}^{m} TII_{ij},
\]

with \(m\) being the total number of species. Next, we calculate the relative TII of each of the target species to the total TII. A large TII thus indicates many trees in close proximity to the subplot, that have the potential to affect the soil chemistry of this subplot. See Supplementary B for a visual representation of the calculation of the TII.
Statistical analysis

To test whether the total C, Olsen P, and base cation (BC) concentration are higher and the pH is lower close to the tree (hypothesis 1), we regressed the concentration or ratio against the log-transformed distance to the nearest tree in the transect. To account for the nested design, i.e. multiple subplots per plot, we used a multilevel model that included plot ID as a group-level (random) effect.

To test whether there exists a species effect on top of the distance effect (hypothesis 2), we performed multilevel models again for each soil variable (C, C:N, Olsen P, pH-H₂O, and BC). As response variable, we subtracted the value observed in the centre subplot from the value observed in the subplot close to a target tree (subplot of interest) and divided this by the value observed in the middle to calculate a percentage difference. We fitted this difference in function of the species identity of the target tree near the subplot and the species identity of the target tree distant to the subplot, to test whether the species identity affects the chemical soil composition. Alongside the main effects of these species identities, we included their interaction effect, to test whether the identity of one tree affects the influence of the other tree in the transect. We also added the logarithmic distance between the subplot of interest and the centre subplot to include the distance effect described in our first hypothesis. Lastly, we included the plot ID as a group-level (random) effect.

Finally, we tested whether we can predict the observations in two-species mixtures based on the constituent species’ monoculture responses (hypothesis 3). For this, we fitted multilevel models using only the monoculture observations. For each target tree species, we fitted an intercept. Once more, we include the logarithmic distance to the nearest tree as a fixed effect and plot ID as a group-level (random) effect. Next, we predicted mixture responses for each subplot in the two-species mixture plots, using the proportional contribution of each of the two target tree species to the subplot’s total TII and the distance to the nearest tree of each transect. We compared these predictions to the observations in the field for these transects, to see whether the predicted pattern deviates from the observed pattern.

The statistical analysis was performed in R version 4.1.0 (R Core Team 2021). We modelled our data with generalized linear mixed models making use of the package MCMCglmm (Hadfield 2010). The model coefficients can be found in the Supplementary C.
Results

Looking at the effect of distance on soil nutrient concentrations, total C, C:N ratio, Olsen P, and BC decreased with the distance from the nearest target tree, whereas the pH is unaffected (Fig 2).

*Fig 2: Changes in concentration or ratio (C, C:N, Olsen P, pH-H_2O, and base cations (BC)) in function of the distance (m) to the nearest tree within the transect. The lines show the model predictions of the concentration or ratio in function of the logarithmic distance and the shading shows the standard deviation. The dots show the observed concentration or ratio for each of the subplots with the colour denoting the location within the subplot (blue: close to a target tree; orange: midway between two target trees). Significance level of the distance-to-tree variable: p-value = <0.001: ***; 0.001: **; 0.01: *; 0.05: n.s.*

The differences in total C, C:N, Olsen P, and BC between the subplot near one target tree and the subplot midway two target trees are mostly positive (blue colour), which illustrates that these chemical concentrations and ratios are larger adjacent to a tree than at a greater distance in most cases (Fig 3). In case of pH, we found the opposite to be true. The difference in pH between the two subplots is mostly negative (orange colour), indicating a lower pH close to a tree. However, different species combinations show differences in strength and in some cases direction of this difference. We highlight an example for the
concentration of Olsen P in a monoculture of oak versus a mixture of oak and birch (indicated by a dashed border in Fig 3 and illustrated in the bottom-right panel). On the diagonal, we see a relatively strong positive effect of the oak species on the Olsen P concentration (number 1). When oak is admixed with birch, we see that this effect is slightly less positive, both in the neighbourhood of oak (number 2) and in the neighbourhood of birch (number 3).

Further, in a monoculture of hornbeam (H), the subplots close to the trees have a larger pH (above 0%) than the centre and a smaller concentration of Olsen P (below 0%) than the centre subplot. We see that mixtures of oak (O) or pine (P) in combination with hornbeam (H) have a greater difference in pH and BC between the centre subplot and the subplot close to the stem, than their respective monocultures. The concentration of BC near oak (O) trees is distinctively larger than the concentration further from the tree in a mixture of oak (O) and pine (P).

**Fig 3:** Predicted percentage difference between the chemical composition (C, C:N, Olsen P, pH-H₂O, and base cations (BC)) of the subplot close to a target tree (subplot of interest) and the subplot in the centre of that same transect. We predicted the percentage difference in function of the tree species of the target tree closest to the subplot of interest (Near species: P = pine, O = oak, B = birch, H = hornbeam) and the other target tree (Distant species) and their interaction effect and the logarithmic distance between the subplot of interest and the centre subplot. Note that the monoculture responses are plotted on the diagonal and have a larger linewidth. The predictions shown in the Fig are of all species combinations in our study (note the absence of the combination birch and pine) and use the mean distance over all subplots. The hue denotes the direction of the difference with blue being positive and orange being negative. The intensity denotes the strength of the difference. Bottom-right: three of the species combinations are
highlighted (monoculture of oak: 1; oak-birch mixture nearby oak: 2; oak-birch mixture nearby birch: 3). The concentration or ratio at the centre subplot is used as baseline, which makes the value 0% in the centre subplot. The slope denotes the difference in chemical composition between the subplot of interest (subplot closest to the near species and shown at the left-hand side of the graph) and the centre subplot (shown in the middle of the graph) and is dependent on the distant species (shown on the right-hand side of the graph) and the near species.

We see that the predictions of the chemical composition along the transects in mixture plots made with models using monoculture observations (blue lines) follow a similar pattern as the observations in mixture plots (grey violin plots) (Fig 4). The figure also shows there are only small differences between the concentrations close to either tree species, both for the predictions and for the observation.

![Fig 4: Chemical variation in mixture transects. The grey violin plots and black dots show the observed concentration or ratio (C, C:N, Olsen P, pH-H₂O, and base cations (BC)), where the left and right data points are observations of the subplots close to the target trees and the centre data points are observations in the centre subplot. The blue lines and the shaded area show the predicted mean and standard deviation based on the monoculture responses. We fitted the monoculture observations in function of the target species and](image-url)
the distance to the nearest target tree. Based on these model results we predicted the expected mixture response using the proportion of the target species and the distance to the nearest tree of the respecting subplot.
Discussion

We investigated the effect of trees on the chemical soil composition in the local neighbourhood in a mature forest. Setting up a transect-based design in monocultures and two-species mixtures of different tree species allowed us to quantify the relative importance of pure distance-to-the-tree effects and species identity effects. We found that the total C concentration, the C:N ratio, Olsen P, and base cation concentration (BC) decrease with an increasing distance to the tree. We also discovered distinct tree species identity effects, but no clear non-additive diversity effects. Below, we first discuss the effect of distance, regardless of species identity or diversity effects. Next, we examine the few identity effects that exists on top of those distance effects. Finally, we explore different explanations for the lack of diversity effects of tree species mixtures on the chemical soil composition in the local neighbourhood.

Strong distance to tree effect

Elemental concentrations differ depending on the distance to the tree (first hypothesis). A decrease in total C and C:N with the distance to the nearest tree can be explained by decreased inputs of organic matter, caused by smaller leaf litter inputs (Ferrari 1996), due to higher litter fall close to the stem (Staelens et al. 2003) and litter accumulation by wind at the base of the tree (Facelli and Pickett 1991), smaller root litter inputs, and reduced microbial activity (Manoharachary and Mukerji 2006) at a greater distance to the tree. A smaller organic matter content further leads to a smaller binding capacity (Jenny 1980), which can explain the smaller concentration of BC and Olsen P at a greater distance to the tree.

We did not find a reduction in pH closer to the stem base, in contrast to what we hypothesized. Previous studies linked an observed lower pH nearby the tree to stemflow (Levia and Frost 2003). The somewhat acidic water is concentrated in a narrow zone around the stem base. In contrast, other studies describe uptake of H+ ions and leaching of BC through the canopy, especially for broadleaved species, causing an increase in pH in the narrow zone around the tree (Adriaenssens et al. 2012; Hamdan and Schmidt 2012; Kowalska et al. 2016; Gautam et al. 2017). Stemflow can, for that reason, be a source of BC as well (Parker 1983), and may partially explain the increase in BC we found (Koch and Matzner 1993). The opposing findings reported on soil acidity in literature can be explained by its dependence on site conditions, like differences in the composition of atmospheric depositions, which effects the composition of the stemflow (Levia and Frost 2003).
Stemflow only contributes to a small extent the total water input to the soil for the tree species considered in our study (André et al. 2008; Nasiri et al. 2012), which can help to explain the lack of distance-to-tree effect on the pH at our site. Moreover Van Stan & Gordon (2018) argue that in many instances stemflow has a limiting effect on soil nutrient availability, if not sufficiently supplemented by throughfall, due to its small volume. Further, it is possible that our sampling was not close enough to the base of the tree to be affected by the stemflow. The zone most influenced by stemflow extends to one square meter around the stem (Chang and Matzner 2000). The zone around that (where we sampled) is mainly affected by throughfall.

Throughfall, which has similar attributes to stemflow, although less concentrated, covers a larger area around the tree (Parker 1983). Depositions in throughfall also show a clear decreasing gradient with increasing distance to the base of the tree (Beier et al. 1993), which can explain the observed effect on BC in our study.

**Small species identity effects**

We found some support for our second hypothesis for which we expect a species identity effect in addition to the distance effects. In general, we showed that for most tree species compositions the difference in concentration between the subplot close to the tree and the subplot in the centre of the transect has the same direction as the overall distance effect, suggesting that the different tree species affect the soil in a similar manner. However, we revealed divergence in the strength and direction for some species compositions. The differences that can be distinguished can be explained by species specific differences, including litter quality and quantity, interception of deposition, root distribution, and nutrient uptake (Augusto et al. 2002).

First, we will describe the distance effects in monocultures (diagonal in Fig 3). We revealed a larger distance effect on C in monocultures of oak, than in monocultures of birch and hornbeam. Vesterdal et al. (2013) reported lower soil organic C concentration in the mineral soil under species with nutrient-poor litter. Although, several studies also found no significant effects. The larger distance effect under oak, a poor-litter species, in our study is contradictory to these findings. The distance effect on C:N is positive in birch monocultures, unlike monocultures of the three other species. The litter quality of birch may explain the difference with pine and oak, but the difference with hornbeam, which has an even better litter quality, is surprising. The soil in the close proximity of the base of the stem in monocultures of oak and pine is more
The soil at a location at a further distance, which demonstrates the acidifying effect of both species, that has been described repeatedly in literature (Neirynck et al. 2000; Van Nevel et al. 2014). For birch monocultures, a species that has been proposed for soil restoration due to its more nutrient-rich litter, we also identified this acidifying effect. This is in contrast to hornbeam, also a species with nutrient-rich litter. Here we see a net increase in pH close to the stem base in monocultures. Studies have shown that hornbeam has a greater litter quality than birch (Hobbie et al. 2006), so it is not surprising that the effects of hornbeam on soil fertility are stronger.

Next to the monocultures, we also found species effects in two-species mixture. If the difference between the subplot close to the tree and the centre would only depend on the distance between both subplots and the identity of the species in the near proximity of the subplot, there would be no difference within a row in Fig 3. We, however, revealed some variation within a row, indicating that the identity of the other target species is likely of importance as well. These identity effects on neighbourhood level are potentially leading to identity effects on plot scale. Indeed, Dawud et al. (2016) reported effects of species identity on chemical properties in the topsoil, however small, in the same study site on plot level.

We found positive effects on soil pH in mixtures of hornbeam with birch or pine (represented by a blue colour in Fig 3), illustrating the restoring capacity of admixing with hornbeam, reported in other studies (e.g. Kooch & Bayranvand, 2017). We discussed the lack of difference in distance effect on the soil pH in pine, oak, and birch monocultures above. This may help explain the absence of ameliorating effect of birch trees in mixtures with pine or oak.

The restoring capacity of admixing with hornbeam is also visible in the availability of BC: the increase near the stem is larger near birch and pine trees in mixtures with hornbeam than in monocultures. Here we also identified an ameliorating effect in close proximity of oak in an oak-pine mixture. Despite the fact that oak is a species with bad-litter quality (Hobbie et al. 2006), it has fast-decomposable litter relative to pine, and has the potential to restore soil fertility in coniferous forests (Polyakova and Billor 2007). The lack of ameliorating effects near hornbeam in hornbeam-pine mixtures (both for pH and BC) is surprising. Other factors that we did not account for may be at play at this small scale, including spatial variations in herbivory or woody debris (Bardgett and Wardle 2003; Ehrenfeld et al. 2005; Dhiedt et al. 2019).
For P, there is a smaller difference in availability between the concentration close to the stem and the concentration at a greater distance in two-species mixtures in comparison to the monocultures in most cases, opposed to what we expected based on their difference in litter quality. Nutrient-rich litters release P at a faster rate. Therefore, we expected a greater availability of P in the mineral soil under these litter types in comparison to nutrient-poor litter (Osono and Takeda 2004; Talkner et al. 2009).

We revealed a small difference between the two distances to the tree in the C:N ratio in hornbeam-pine and birch-oak mixtures. A lower C:N ratio is linked to higher soil fertility (Galloway et al. 2004), further supporting our hypothesis that the nutrient-rich litter species (hornbeam and birch) can restore soil fertility at a small scale in stands with nutrient-poor species. There is a similar effect in a mixture of oak and pine. Similarly to our results for BC, we can argue that oak, being a broadleaved species, is a species with higher litter quality relative to pine (Sariyildiz et al. 2005; Polyakova and Billor 2007), although this difference may not be very large (Hobbie et al. 2006).

We mainly found positive effects of admixing high-quality litter species with oak and pine in the case of hornbeam and less so for birch. The litter quality of hornbeam is higher (Hobbie et al. 2006) and may have a stronger impact on the topsoil chemistry. It is important to mention that the plots we describe as “monoculture” are not pure monocultures (the admixture ranges from 5% to 40% of the total basal area in monocultures). This can explain the fact that the soil in the pine and oak plots is not strongly degraded and the lack of positive effects of admixing birch. Moreover, the shrub layer in most plots consists largely of hornbeam. Czerepko (2004) hypothesized that the development of a second canopy layer with hornbeam prevented soil degradation in pine stands in a similar habitat. Despite the fact that the shrub layer at our site consist largely out of hornbeam, we see that locally, admixing even more trees with nutrient-rich litter can still have beneficial effects on soil fertility. Desie et al. (2020) reported that increasing the share of nutrient-rich litter in a forest stand can enhance soil restoration. This demonstrates that increasing the proportion of trees with good-quality litter, indeed, has the potential to further improve the soil fertility and explains why we found positive effects of mixing hornbeam in our stands that already are admixed with hornbeam, but to a lesser extent.

No species diversity effects
The predictions based on the monoculture responses (blue lines) do not greatly deviate from the observed data (grey violin plots) (Fig 4). From this, we may deduce that there are no considerable diversity effects at play, in contrast to what we hypothesized (hypothesis 3). Dawud et al. (2016) concluded that differences in the topsoil between plots at this site were driven by purely additive species identity effects, rather than diversity effects, similar to studies elsewhere (e.g. Osei et al., 2021; Zhang et al., 2018). Our results show that also on a smaller neighbourhood scale, these diversity effects cannot be found and are likely very subtle or even non-existent.

The lack of diversity effects can be explained by several mechanisms. First, a major source of soil C is linked to root biomass, more specifically a greater root biomass causes greater litter and exudates inputs (Bardgett et al. 2005). Peng & Chen (2021) have shown that diversity effects on root biomass strengthen with the depth of the soil. In our study, we are only looking at the first 10 cm of the soil, where diversity effects on root biomass are marginal. Indeed, Chen et al. (2020) observed stronger diversity effects on soil C in deeper soil layers, as did Dawud et al. (2016) in the site used in this study.

Second, this study made use of an exploratory platform. Although plots were selected carefully to minimize differences in management and other factors between plots and species compositions (Baeten et al. 2013), working in an existing forest rather than a common garden experiment always entails the risk of unwanted covariation with environmental factors that affect the soil. Alongside these potentially varying factors between plots, we also have to address within-plot variability, since we are comparing different locations within a plot. Also on this level, there may be factors at play that we are not considering, like legacy effects of previous generations (Cuddington 2011). Indeed, it has been shown that effects on the chemical soil composition of trees that are cleared or coarse woody debris can persist for a long time after these trees were felled or debris has been broken down (Trumbore 2000). This can cause spatial variability that may interfere with the spatial variability we expect based on the current vegetation.

For a third potential explanation, we look at the species composition. Osei et al. (2021) detected diversity effects on soil organic carbon in the topsoil, but only for one of the three tree species combination they studied. Similarly, they found diversity effects only for one composition in deeper soil layers. They suggested that the existence of diversity effects is likely dependent on species composition and their interspecific
competition, their associated soil biota, and the environment. Although the species considered in our study are functionally dissimilar, including in litter quality and root architecture, they may potentially not interact in a way that is sufficient to create diversity effects on the soil. For the combination oak and pine for example, Osei et al. (2021) found no diversity effects on soil organic carbon, comparable to our results.

Fourth, soil type can affect the strength of the influence of species mixing on the soil (Verstraeten et al. 2018). Desie et al. (2020) relate the strength of mixing effects to clay content and soil buffering. They argue that increasing clay content may lead to stronger mixing effect, due to a greater SOM stabilization, aggregation, weatherable reserve, and water holding capacity. When the clay content is high, however, it may prevent changes to soil acidity, due to a large buffering capacity. Our study plots are situated on cambisols and luvisols, which are reasonably fertile and may buffer mixing effects to a large extent.

Conclusion

Our results show a strong effect of distance to the tree on the soil chemistry, creating variability within a stand. Further, we showed that on this small neighbourhood scale, species identity plays a role in determining the chemical composition of the topsoil. However, we did not find any evidence supporting additional diversity effects. These results suggest that we can predict the chemical composition of the soil in mixtures by aggregating monoculture effects. Although we did not find positive diversity effects on the chemical soil composition, like increased carbon concentration or greater availability of BC, we also did not find any negative effects. However, our results do highlight the importance of species choice to topsoil chemistry. We showed evidence in favor of the management strategy of admixing species with high-quality litter, like hornbeam, to ameliorate soil fertility on a local scale in forest stands consisting of economically important species with often low litter quality, like Scots pine and pedunculate oak, strengthening the support for mixed forests.

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**Competing interest**

The authors declare no competing interest.

**Authors’ contributions**

E.D., L.B., P.D.S., and K.V. conceived the conceptual idea and outline. B.J. maintains the observational site. E.D. performed the statistical analysis and wrote the original draft. L.B., P.D.S., B.J., and K.V. reviewed and edited the manuscript.

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