Title

Little effect of tree species richness on within- and between-plot variability in soil chemical properties in a young plantation forest.

Running title

Species richness effect on spatial soil variation

Author names

Els Dhiedt^{a,1} (els.dhiedt@ugent.be; +32 9 264 90 37)

Lander Baeten^a (lander.baeten@ugent.be)

Pallieter De Smedt^a (pallieter.desmedt@ugent.be)

Kris Verheyen^a (kris.verheyen@ugent.be)

^aForest & Nature Lab, Department of Environment, Faculty of Bioscience Engineering, Ghent University, Geraardsbergsesteenweg 267, 9090 Melle-Gontrode, Belgium

¹corresponding author

Abstract

Trees impact the chemical properties of the soil in which they grow. When planting forests, the choice of tree species and species combinations is thus expected to partly determine the spatial variation in soil properties, even in the early stages of forest development. We made use of a biodiversity-ecosystem functioning experiment in Belgium, FORBIO. The studied site is situated on former agricultural land on loamy sandy soil and replicates tree species richness (1 to 4 species) and composition. Soil samples (0-10 cm) were taken at five locations in 1, 2, and 4 species plots. We measured the total C and N concentration, the Olsen P, the pH-H₂O and the available Ca, Mg, and K concentration.

We hypothesized that the within-plot spatial variability would increase with species richness and that the between-plot variability would decrease with species richness. We found little evidence to support our hypothesis. We only found a smaller within-plot variability in monocultures than mixtures for Ca, pH, and C:N. Potential reasons for the little evidence include the fact that the forest is only nine years old, the

agricultural land-use history and soil texture. Further research in sites with different soil properties or different land-use history is needed to generalize these results.

Key words

Afforestation, base cations, carbon, FORBIO, forest soil chemistry, Olsen phosphorus, pH, tree species diversity, TreeDivNet

Highlights

- Does tree species richness affect spatial variation of soil elements in a young plantation?
- We expect a larger within- and a smaller between-plot variation in mixtures than monocultures.
- We only found a larger within-plot variation in mixtures for Ca, pH, and C:N.
- Little evidence can be result of the young age, agricultural land-use history, or soil texture.

Text

Introduction

Trees have a strong influence on the environment in which they grow. Therefore, important changes can be expected due to afforestation, including in the soil. This effect is influenced to a large extent by the identity of the planted tree species, since tree species typically differ in productivity, litter quality and quantity, and canopy structure (Augusto et al., 2002), leading to differences in water and nutrient uptake, litter decomposition, and microclimate. Planting trees can lead to changes in the soil composition, depending on the species (e.g. Bárcena et al., 2014; De Schrijver et al., 2012). In a previous study (Dhiedt et al., 2021), we took a closer look at how soil properties change over time, as young plantation forests establish and grow. We found clear trends for most elements, but the direction was determined by the site context, including land-use history, site preparation, and soil texture. In this study, we were interested if these temporal differences in soil chemistry result in spatial patterns.

When planting different tree species in mixtures, the spatial resource heterogeneity (Rosenzweig, 2010), is expected to become larger compared with monospecific plantations. Previous studies found effects of mixing species (Andivia et al., 2016) and spatial distribution of species (Sercu et al., 2019) on the spatial

heterogeneity in mature forests. When we look at a larger scale, i.e. the variation between forest stands, we expect that the effects of the different species in mixed stands averages out to a more similar mean over different compositions. Consequently, the variation between mixed stands will be smaller than between monoculture stands. In its turn, the variation in soil nutrients affects the soil microbial (Eisenhauer, 2016; Hooper et al., 2000) and faunal community (Vanbergen et al., 2007), plant establishment and growth (Chesson, 1986). Hence, it is important to study the factors that can affect the spatial variation of the soil chemical composition.

In our study, we looked on what spatial scale topsoil nutrient heterogeneity occurs in monocultures versus mixtures in a young tree plantation on former agricultural land. We hypothesise that the within-plot variability will be relatively small and the between-plot variability will be relatively large in monospecific plots. In contrast, we expect the within-plot variability to be relatively large and the between-plot variability relatively small in mixtures. A schematic representation of our hypothesis is given in Figure 2 (top left).

Materials and Methods

Experimental sites

The study was conducted in a large tree diversity experiment in Belgium (FORBIO, FORest BIODiversity and Ecosystem Functioning (Verheyen et al., 2013)), which is part of a larger network of tree diversity experiments, TreeDivNet (Verheyen et al., 2016; treedivnet.ugent.be). The FORBIO experiment is a multi-site experiment that replicates its full design across three sites. Here we focus on one of the sites, situated in Zedelgem, Belgium (51°9'N 3°7'E). The site is situated on relatively dry sandy soil (Albic Podzol (Arenic)) to moderately wet loamy sandy soil (Gleyic Terric Cambisol (Loamic)) (IUSS Working Group WRB, 2015) and is a former agricultural field, where arable crops (potatoes and maize) were grown. It was ploughed prior to the tree planting. The site-level design of the experimental plantation follows a classical synthetic community approach, i.e. it was planted with five species (*Betula pendula*, *Fagus sylvatica*, *Pinus sylvestris*, *Quercus robur*, and *Tilia cordata*) in different tree species richness levels (1 – 4 species). All species are well adapted to the local environment, functionally dissimilar, and of importance for forest management in Belgium. In total twenty treatments were established: all five monocultures, all five possible four-species mixtures, and

a random selection of five two- and five three-species combinations. These were all replicated once, resulting in 40 plots. Each plot is 42 by 42 meter in size. Trees are planted on a 1.5 by 1.5 meter grid. In mixed plots, trees were planted in small monospecific patches of 3 x 3 trees. Patches were arranged in a checkerboard pattern in the two-species mixtures and randomly mixed in the three- and four-species mixtures (Figure 1). More information can be found in Verheyen et al. (2013).

Soil sampling

In February 2019, we have taken five soil samples per plot, up to 10 cm deep, using a soil auger with a diameter of 3 cm, and after removing the forest floor. We targeted all the monoculture and the two and four species mixture plots. A consistent sampling design was applied in each plot, focusing on the center of the plot (Figure 1). In monoculture and two-species plots, a square of four patches of 3 x 3 trees was chosen. In four-species plots, a random square of four patches of different species was located. In each of these squares a sample was taken at five locations: in the middle of each of the four patches and one in the middle of the square, i.e. the middle of the four patches.

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, 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). Samples were analyzed for pH-H₂O by shaking a 1:5 ratio soil/H₂O mixture for 5 min at 300 rpm and measuring with a pH meter Orion 920A with pH electrode model Ross sure-flow 8172 BNWP, Thermo Scientific Orion, USA. Bioavailable P which is available for plants within one growing season (Gilbert et al., 2009) was analyzed 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). Exchangeable K⁺, Ca²⁺, Mg²⁺, and Al³⁺ concentrations were measured by atomic absorption spectrophotometry (AA240FS, Fast Sequential AAS) after extraction in 0.1 M BaCl₂ (according to NEN 5738:1996). All Al concentrations were under the determination limit of 100 mg kg⁻¹ and hence not further analysed in this study.

Statistical analysis

We related the measured soil variables to the tree species richness (SR) using multilevel distributional models. Such models allow to relax the assumption that the scale parameters (i.e. variances) are constant across observations. Here we fitted a model where the variances are predicted by the tree SR level, both at the between-plot and within-plot scale. Technically, this was done through a group-level (random) effect for plot number and with these between-plot variances dependent on the plots' SR levels. As a result, we can predict the between-plot variance for monocultures, two, and four species mixtures separately (e.g. $\sigma_{between, SR=1}$ for variation between monocultures). Similarly, the residual variation of the model corresponds to the variation between the five samples in a plot and was also considered to be dependent on the plot's SR level, i.e. we can predict within-plot variance for monocultures, two, and four species mixtures (e.g., $\sigma_{within, SR=1}$ for variation within monocultures). We also included block number as a random variable, because both blocks have a different land use history and soil type and hence a different chemical composition of the soil.

Additionally, we calculated the intraclass correlation (ICC) by dividing the squared between-plot variation by the squared total variation (the sum of the between-plot, within-plot, and between-block variation). The ICC describes the strength of the resemblance of different observations within a group and provides us with an insight in the relative importance of the between-plot variation compared to the total variation.

All models were fitted with the probabilistic programming language Stan through the *brms* package with R version 4.0.2 (Bürkner, 2017; R Core Team, 2020). We used the default priors for these multilevel models and ran three chains of 1500 iterations each, after a warm-up of 1500 iterations.

To test our hypothesis, we sampled a thousand times out of the posterior distributions of $\sigma_{between}$, σ_{within} , and the ICC of the different SR levels. For each pair of two SR levels we calculated the difference. Then we evaluated to what degree this difference differs from zero. The p-value is defined as the proportion of differences that is smaller or larger (depending on our hypothesis) than zero. For the within-plot variation we calculate the proportion of samples of which the difference between SR level 1 and 2, as well as 1 and 4, and 2 and 4, is smaller than zero. For the between-plot variation and the ICC, we calculate the proportion of

samples of which the difference between those same SR level pairs is larger than zero. Subtracting that proportion from one gives us the p-value.

Results and discussion

Both the between-plot variation and the within-plot variation of soil chemical properties are similar in the different species richness levels (Figure 2, Table 1). Only for Ca concentration, pH, and C:N ratio, the within-plot variation is smaller in monocultures than in mixtures. Although for the pH this is only the case for two-species mixtures. Likewise, the ICC does not differ among the different richness levels, except for the C:N ratio and only for two-species mixtures.

Except for the soil properties mentioned above, the results show little to no evidence in support of our hypotheses. First, we'll discuss the elements for which we do detect the expected patterns. Then, we elaborate on various reasons that may explain the unexpected patterns of spatial variation in relation to the tree species richness of the plots.

The within-plot variation increases with species-richness level for Ca. Differences in available Ca in the soil are caused by differences in inputs (including weathering of minerals, mineralization of organic material, and leaching from plant biomass) and outputs (including leaching to ground water and plant uptake) (Schlesinger, 1997). These mechanisms are affected by the trees. In monocultures, these tree effects are relatively homogeneous across the plot. In contrast, when multiple species are growing together, different species will impact these mechanisms in various strengths and directions. Hence, the tree effects will be more heterogeneous across the plot. In Dhiedt et al. (2021), we also found a strong negative temporal effect for Ca in this site, likely due to plant uptake and leaching. However, the composition had only little effect on this temporal change. We would expect a similar pattern for K, as this is also a mobile element, which is impacted by similar mechanisms. We however did not find this in our results.

A first explanation of the little evidence can be found in the age of the experiment. The study site where we conducted our experiment was only afforested for nine years at the time of sampling. A possible consequence could be that the different trees have not yet affected the soil during that time, at least not in sufficiently different ways to be picked up by our sampling design. In a previous study at this site (Dhiedt et al., 2021),

we have shown that the overall chemical composition on site level has changed to a great extent since planting. More specifically, we detected an increase in total C, total N, Olsen P, and available K, a decrease in pH and available Ca and Mg, and no significant change in the C:N ratio. But our results suggest that establishment of the expected higher spatial variation in the soil of mixtures may take a longer time. Likewise, we did not find strong composition effects in this site (Dhiedt et al., 2021). Previous studies did not find species effects on available Ca, C:N, and pH in the topsoil during the first decennium after afforestation on agricultural soils (Compton et al., 1998; De Schrijver et al., 2012; Ritter et al., 2003). Without these species effects, we would not expect a difference in within- or between-plot variation between the different SR levels. Even in more mature forests, that have grown over multiple decennia, the effects of SR on the soil nutrient composition have been found to be subtle (Dawud et al., 2016; Guckland et al., 2009; Schleuß et al., 2014; Schmidt et al., 2015).

The experiment is planted on former agricultural land. Fraterrigo et al. (2005) found a long persistence of former land-use effects on spatial heterogeneity of soil chemical composition. The fact that homogeneity caused by agricultural activities can persist for a long time, can explain the lack of evidence we found.

In forest planted on agricultural soil, it is expected that before crown closure there would be a difference in soil composition between the zones under a tree, where roots would have taken up nutrients and litter would have been accumulated, and the zones between the trees, where the tree effect would not have reached yet. This variation would exist even when all trees are of the same species. Plots containing slow growing late successional species reached this stage in forest development only shortly before sampling. This variability is likely not picked up by our sampling scheme, which does not sample at multiple distances from the trees.

Another potential reason can be found in the soil properties. The experiment is situated on loamy sandy soil. This relatively coarse texture allows for a greater mobility of nutrients within the soil in comparison to soils with a fine texture (Lehmann & Schroth, 2003). Hence, a greater homogenization can be expected within a plot, which could explain the lack of difference in spatial variation between the monocultures and mixtures. On the other hand, due to a low buffering capacity, significant effects can happen at a faster rate in coarse textured soils (Parfitt et al., 1995). This would suggest that the process would take even longer in fine textured soils.

Conclusion

In this study, we found little evidence to support our hypothesis that within-plot variation in soil chemical composition increases and between-plot variation decreases with species richness. We have shown that species richness does not impact the between-plot variation of soil elements and does impact within-plot variation of only certain elements in this young plantation on former agricultural land on loamy sandy soil. The lack of differences could be explained by the fact the plantation is only nine years old and by the soil properties of the site. Further studies on sites with a finer soil texture or different land-use history are necessary to be able to generalize for different soil conditions.

Conflict of interest

The authors declare no conflict of interest.

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Tables

Table 1: P-values comparing the posterior distributions of the variations and the intraclass correlations (ICC) presented in Figure 2. P-values smaller than 0.1 are highlighted in bold.

Nutrient	C	C:N	Olsen P	pH-H ₂ O	Ca	K	Mg
$\sigma_{within,SR=1}$ < $\sigma_{within,SR=2}$	0.707	0.076	0.806	<0.001	0.068	0.801	0.668
$\sigma_{within,SR=1}$ < $\sigma_{within,SR=4}$	0.571	0.069	0.942	0.643	<0.001	0.932	0.293
$\sigma_{within,SR=2}$ < $\sigma_{within,SR=4}$	0.353	0.501	0.729	0.998	0.084	0.769	0.143

$\sigma_{between,SR=1}$ $> \sigma_{between,SR=2}$	0.394	0.068	0.155	0.484	0.866	0.644	0.771
$\sigma_{within,SR=1}$ $> \sigma_{within,SR=4}$	0.925	0.596	0.384	0.854	0.810	0.291	0.888
$\sigma_{between,SR=2}$ $> \sigma_{between,SR=4}$	0.948	0.961	0.759	0.840	0.396	0.168	0.697
$ICC_{SR=1}$ $> ICC_{SR=2}$	0.403	0.052	0.222	0.439	0.806	0.700	0.787
$ICC_{SR=1} >$ $ICC_{SR=4},$	0.918	0.470	0.499	0.853	0.630	0.418	0.834
$ICC_{SR=2} >$ $ICC_{SR=4}.$	0.945	0.961	0.796	0.858	0.314	0.228	0.615

Figures

Figure 1: Sampling design of the (a) monoculture, (b) two species, and (c) four species mixture plots. Each coloured square represents a small monocultural patch of three by three trees. Each black dot represents a location where a sample is taken.

Figure 2: Per element or ratio: (Left) the median of the posterior distribution of the between-plot variation is plotted along the x-axis and the median of the posterior distribution of the within-plot variation is plotted along the y-axis for the three species-richness (SR) levels. The standard deviation on these variations is indicated by error bars. (Right) The posterior distribution of the intraclass correlation (ICC) for the three SR levels. The median is represented by a vertical line in the respective colours. The first panel (top left) shows the model results of our hypothesis, the other panels show the model results of the observed data.

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