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Mixed-Species Effects on Soil C and N Stocks, C/N Ratio and pH Using a Transboundary Approach in Adjacent Common Garden Douglas-Fir and Beech Stands

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Abstract: Mixed forest of Douglas-fir and beech has been suggested as one of the possible future forest types in Northwest Europe but the effects of this mixed forest on soil properties relative to monoculture stands are unknown. In a transboundary investigation of adjacent common garden Douglas-fir and beech stands, we determined the effects on topsoil properties. However, responses of C and N stocks, the C/N ratio and pH were site- and soil layer-specific and were mainly single-sided and without synergistic effects. Beech reduced the soil C and N stocks in Douglas-fir at the nutrient-poor site, caused an increase in the C/N ratio in the forest floor and mineral soil at both nutrient-poor and -rich sites, and reduced the acidifying effect of Douglas-fir at the nutrient-poor site. These results do not support the hypothesis that mixture effects would be consistent across sites and soil layers. The lack of synergistic effects may be attributed to the relatively similar litter quality or rooting depth that prevented any larger niche differentiation and complementarity. The results indicate that the transboundary approach within a mature common garden proved useful as a platform to test tree species interactions, and this approach could be explored in soil studies until dedicated mixed-species common gardens reach maturity.

Keywords: mixed-species forests; Douglas-fir; European beech; soil C stock; soil nutrient status; transboundary approach

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1. Introduction

It has been suggested that mixtures of tree species rather than monocultures would better support the long-term nutritional sustainability of forests and provision of ecosystem services including productivity [1–3] and adaptation to climate change [4,5]. Therefore, forest management is faced with the challenge to identify relevant species for such mixed forests. Candidate species for future mixed stands, for instance in central and northern Europe, may include both indigenous and introduced species. One of the introduced species in this region is Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) that is native to Northwest America and Canada [6,7]. Douglas-fir has been viewed as a promising species to European forestry owing to its fast growth, valuable timber and most importantly because of its adaptability to the ecological and climatic settings of the region [8]. It also grows well together with other conifers and broadleaved species [3]. These characteristics have made Douglas-fir a potential tree species for mixtures with native species such as European beech (*Fagus sylvatica* L.) [9,10].

Mixed stands of Douglas-fir and beech are currently established by replacing pure stands of Norway spruce (*Picea abies* L. Karst) or Scots pine (*Pinus sylvestris* L.) [10] or by introducing Douglas-fir into existing beech stands or vice versa [11]. The mixed forests are believed to be beneficial with regard to adaptation to climate change and delivery of various ecosystem functions and services that will meet the demand for multifunctionality [11,12]. However, the more exact responses of forest ecosystem functions and services to this change in management are not yet fully explored and documented. Specifically, how mixtures of Douglas-fir and beech influence forest soil nutrient status and soil C sequestration and nitrogen retention are some of the issues at stake.

Species mixtures have not shown consistent effects on foliar nutrition when compared to the pure stands [3]. Transformation of pure stands of spruce and Scots pine into mixed stands of Douglas-fir and Norway spruce or Scots pine increased soil N stocks but did not affect the soil organic C stock in Germany [10]. Based on a neighborhood design, Rothe et al. [13] concluded that Douglas-fir mixed with red alder, and beech mixed with Norway spruce did not improve the foliar nutrition of the conifers. However, it was documented that mixed stands of Douglas-fir and red alder have a synergistic effect on soil C and N pools compared to pure stands that was attributed to the N-fixing capacity of red alder [14–16].

Studies of forest soil properties under monoculture stands of beech and Douglas-fir showed that forest floors had lower pH under Douglas-fir than beech [17,18]. Results were more inconsistent for C and N stocks and the C/N ratio. There have been reports of similar forest floor C/N ratios [18], higher C/N ratios in beech than in Douglas-fir stands [19] and vice-versa [17]. It was also reported that Douglas-fir stands had larger forest floor mass [19] and higher forest floor C content than beech monocultures [20], but other studies showed that the two species have comparable forest floor C and N stocks [18]. This inconsistent information concerning the effects of the pure stands suggests that even in case of additive effects, there would be limited support for firm conclusions on how mixed stands of beech and Douglas-fir would affect forest soil C, N and pH. This limited information on the effects of mixed stands could be attributed to the lack of mature mixed stands of the two species because studied stands were so young that changes in soil C and N stock could not be detected [21] and the general lack of experimental designs to address mixed species effects on soils in a comparable way to that of single species [15]. Even though information is lacking about the effects of the mixed stands of beech and Douglas-fir, studies that used data from existing forests showed that species mixtures may increase soil C and pH [22–24].

In this study, we investigated how forest soil properties of mixed stands of beech and Douglas-fir could be drawn from a transboundary approach in adjacent monoculture stands of the two species in mature common garden experiments as suggested by Binkley [25] and so far mainly used in studies of mixed stands having N-fixing and non-N fixing tree species [15,16]. In the transboundary approach, the transition zone around the interface of the two stands approaches conditions of a mixed stand of the two species, and the interior part of the pure stands represent conditions of a single species stand. In common gardens, the confounding effect of site factors are minimized by the design [26]. We investigated forest soil C and N stocks, the C/N ratio and pH across the boundary of neighboring stands of Douglas-fir and European beech with the objective to test whether the boundary (species mixture) will exhibit synergistic or additive effects on soil properties. A synergistic effect in the transboundary approach would be characterized by a double-sided, unidirectional effect on soil properties toward the stand boundary. We tested the following hypotheses: (1) monocultures of beech and Douglas-fir have similar C and N stocks, C/N ratios and pH in the soil; (2) mixtures of beech and Douglas-fir show synergistic effects compared to the respective monocultures in C and N stocks, C/N ratios and pH; and (3) the mixture effects are consistent across sites and soil layers.

2. Materials and Methods

2.1. Site Descriptions

The study was carried out at two Danish common garden sites where Douglas-fir and beech stands were placed adjacently with no forest tracks or disturbances interfering with the boundary zone. The Christianssæde (CHR) site is on the island of Lolland in southern Denmark (54°47′ N, 11°22′ E) [27].

It has a mean annual temperature of 8.1 °C (average for 1964 to 1998), a mean annual precipitation of 600 mm and a precipitation deficit in the growing season of 147 mm. CHR has a nutrient rich soil developed from loamy weichselian till and is classified as a Mollic Hapludalf [28]. The parent material contained up to 28% $CaCO_3$ in the C-horizon (Table 1) and the soil is imperfectly drained [29]. The other study site, Løvenholm (LØV), is located in eastern Jutland (56°28′ N, 10°32′ E) and is more nutrient poor compared to CHR [18]. The mean annual temperature is 7.4 °C (average for 1964 to 1998), the mean annual precipitation is 613 mm and the precipitation deficit in the growing season is 116 mm. The LØV soil is moderately drained [29] and the soil is classified as a Typic Haplumbrept [28] with a loamy sandy texture [18]. The soil of LØV is more acid and has less clay and exchangeable cations (Ca^{2+}, Mg^{2+} and K^+) than CHR. LØV has a higher content of extractable P in the A and Bt horizons than CHR. Both sites were agricultural land until they were afforested with the common garden species experiment in 1964 [18]. The common garden stands at both sites were 47 years old at the time of sampling and each stand had an area of approximately 0.25 ha [30] (Table 2).

Table 1. Soil properties at Christianssæde (CHR) and Løvenholm (Løv) sites (adapted from [18]).

Horizons	Depth (cm)	pH	Clay (%)	Silt (%)	Fine Sand (%)	Coarse Sand (%)	Total C (%)	Total N (%)	Extractable P ($mg \cdot kg^{-1}$)	Exchangeable Cations ($cmol \cdot kg^{-1}$)		
										Ca^{2+}	Mg^{2+}	K^+
CHR, Mollic Hapludalf, loam												
A1	0–5	3.8	12.0	10.0	49.1	28.9	2.8	2.1	110	4.60	0.60	0.12
A2	5–25	5.2	12.0	11.0	50.2	26.8	1.5	1.7	110	9.30	0.40	0.10
Bt	25–50	6.2	15.0	18.0	43.6	23.4	0.4	0.5	240	12.20	0.60	0.18
Btg	50–73	7.5	18.0	20.0	38.5	23.5	-	-	380	21.70	0.50	0.13
Ckg	73–110	7.7	9.0	16.0	52.6	22.4	-	-	-	-	0.30	0.07
LØV, Typic Haplumbrept, loamy sand												
Ap	0–27	4.1	3.5	12.0	36.8	47.7	0.9	0.7	119	0.47	0.06	0.06
Bw	27–55	4.8	2.5	6.5	49.0	42.0	0.4	0.3	161	0.48	0.05	0.04
BC	55–70	4.7	4.0	9.0	48.7	38.3	0.1	0.2	223	0.21	0.03	0.04
Cg	70–	4.5	3.5	10.5	43.6	42.4	0.1	-	123	0.78	0.07	0.07

For explanation of terminologies of the soil sub-horizons, please refer to [28].

Table 2. Stand data from 2013 for the investigated stands.

Species	Stem Number ($N \cdot ha^{-1}$)	$Height_{100}$ (m)	Basal Area ($m^2 \cdot ha^{-1}$)	Volume ($m^3 \cdot ha^{-1}$)	MAI ($m^3 \cdot ha^{-1} \cdot year^{-1}$)
CHR					
Beech	362	24.2	23	324	12
Douglas-fir	583	30.2	57	725	19
LØV					
Beech	275	25.3	21	304	10
Douglas-fir	334	31.2	43	570	23

$Height_{100}$ is the mean height of the 100 trees with the largest diameter and MAI is the mean annual increment from seed including thinnings.

2.2. The 'Transboundary Approach' and Experimental Design

The transboundary approach was first suggested by Binkley [25] and, to the best of our knowledge, has since then only been used for a few other studies [15,16]. The boundary where the two-species meet can be interpreted as a mixture proxy. The asset of the transboundary approach is that hypothetically effects may decline by the distance from the boundary and into the monoculture (Figure 1). Systematic evaluation of potential mixture effects can be made along the transect from the one monoculture via the boundary (mixed stand) and into the other species. In Figure 2, we present a conceptional view of the potential mixture effects based on the transboundary approach (no mixture effects with and without species differences, additive effects, single-sided effects and syn- and antagonistic effects).

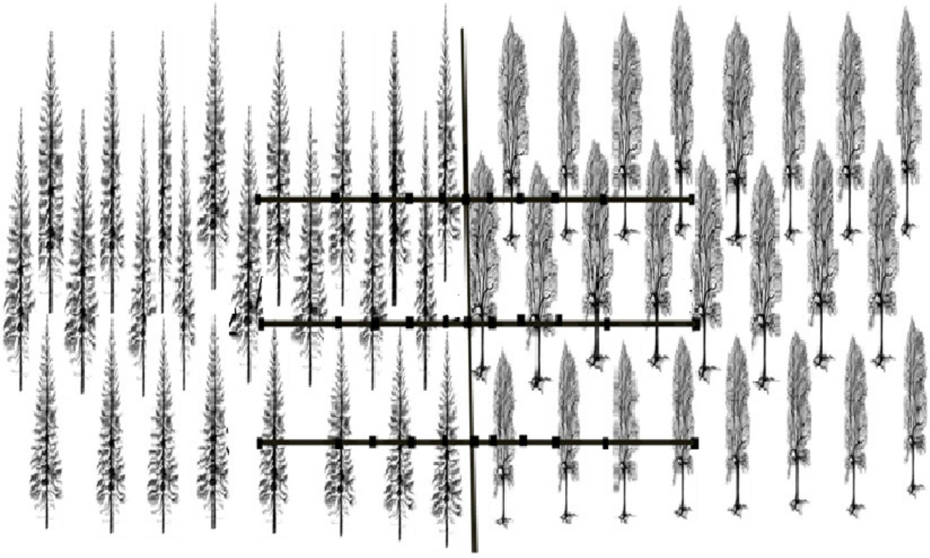

Figure 1. Sketch of the transboundary experimental design. The three transect lines run from the center of the Douglas-fir plot (18 m from the boundary) to the boundary and then to the center of the beech plot (18 m from the boundary).

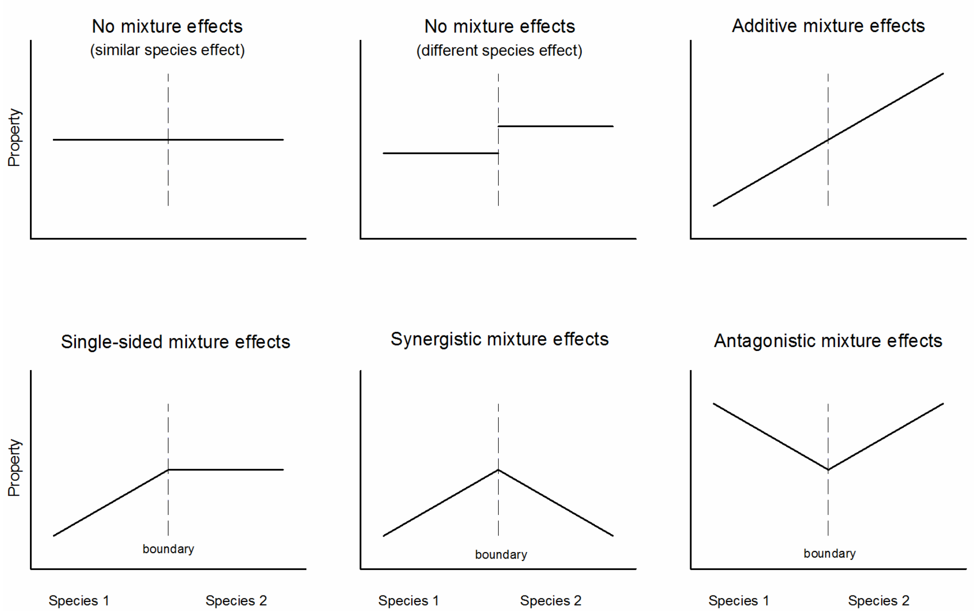

Figure 2. Conceptual graphs showing possible mixture effects in a transboundary design in adjacent common garden stands of two tree species.

Three transects were laid out between the monoculture end of the Douglas-fir plots via the boundary and into the beech monoculture end at both sites (Figure 1). On each transect, 11 points were marked and sampled (0, 1 m, 3 m, 6 m, 10 m and 18 m on both sides) with reference to the boundary set as zero-meter distance (Figure 1). Forest floor (FF) and mineral soil were sampled at each point using a 25 cm × 25 cm wooden frame and a 4.5 cm internal diameter soil corer [31] down to 20 cm depth, respectively. The mineral soil samples were further divided into 0–5 cm, 5–10 cm and 10–20 cm fixed depths.

2.3. Sample Preparation and Laboratory Analyses

The forest floor and mineral soil samples were oven-dried at 55 °C to constant weight before any further laboratory processing and analyses. The forest floor samples were sorted by species and then by identifiable foliar, non-foliar and non-identifiable humified fractions of the litter. The three different forest floor fractional samples were separately ground with a Heavy-duty Model SM 2000-Retsch cutting mill (Retsch, Germany) and a subsample from each fine fraction of the three groups was further ground for ten minutes with a ball mill. The mineral soil samples were passed through a 2-mm diameter sieve. Subsamples from the fine soil fractions (<2 mm diameter) were ground with a Retsch mortar grinder RM 200 (Retsch, Germany) for 10 min. Another subsample was oven-dried at 105 °C to correct for moisture content.

Carbon and N concentrations of the forest floor fractions and the mineral soil layers were determined based on the dry combustion method [32] using a Thermo Scientific FLASH 2000 soil CN analyzer, Italy. Soil pH was measured in 0.01 mol $CaCl_2$ suspensions at ratios of 1:10 and 1:2.5 for organic materials and mineral soils, respectively, with a Radiometer combination-electrode GK2401 (Radiometer, Copenhagen, Denmark). Soil pH values in all layers were lower than 6.0 indicating no presence of carbonates [33,34]. We also performed a fizz test with 4N HCl drops to confirm that the soil samples were free of carbonates. Based on these evaluations, the C content was considered to be of organic origin.

2.4. Calculations of Stocks and Statistical Analyses

We estimated the soil bulk density from the oven-dried and moisture corrected (105 °C) fine soil mass and its volume. The fine soil volume was estimated from the difference between the volume of the soil corer and the volume of stones and roots. Carbon and N stocks in each soil layer were estimated from the soil bulk density, concentrations of C and N and depth of the soil layer. Carbon and N stocks in the entire studied soil profile were summed and hereafter referred to as FF + 0–20 cm. The C/N ratio was calculated for each layer and the FF + 0–20 cm.

We defined multiple linear regression models with both continuous and categorical variables. Species (beech and Douglas-fir), sites (CHR and LØV) and transects (three transects nested within sites) were categorical variables and the distance (0 to 18 m on both sides, Figure 1) was a continuous variable. The analyses were carried out in three steps: (1) in an overall model (global model), we investigated the effects of distance, tree species, sites, transects and interaction of distance with the site (distance: site) and species (distance: species) by soil layers to test site level responses for the mixture effect and its difference between sites and between species (Tables S9–S12); (2) we then examined the main and interaction effects of distance and species by soil layers within each site (Tables S1–S8); Non-significant interactions were removed [35] and (3) given the significant interactions, we finally tested the slope (different from zero) by species, site and soil layers (Figure 3A–D, Figure 4A–D and Figure 5A–D).

The statistical package R version 3.1.0 was used for all analyses [36]. Trends (linear and polynomial) were tested using the lstrends function from the least-square means (lsmeans) R package [37]. The proportion of variance explained by each of the explanatory variables was calculated by partitioning the R^2 using the *calc.relimp* function from the *relaimpo* R package and the *lmg* (Lindeman, Merenda and Gold) metric that partitioned R^2 by averaging over orders [38,39]. Whenever necessary, we log transformed the dependent variables to meet assumptions for linear models such as normal distribution of residuals and homogeneity of variances. In all analyses, we used the 5% significance (α) level.

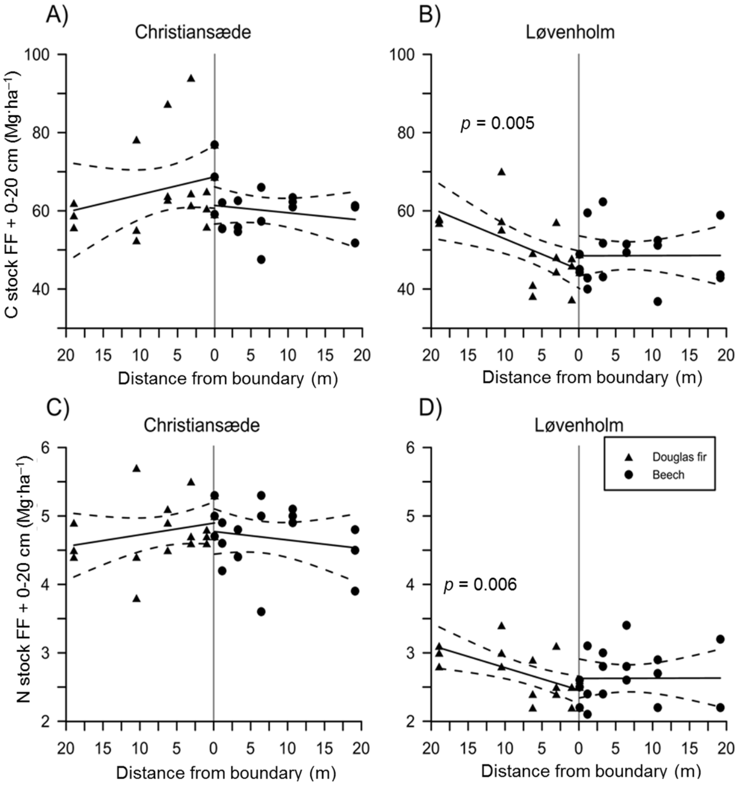

Figure 3. Response of the C stocks (**A** and **B**) and N stocks (**C** and **D**) in the forest floor (FF) + 0–20 cm layer along transects from pure Douglas-fir and beech stand conditions to the boundary at Christianssæde and Løvenholm.

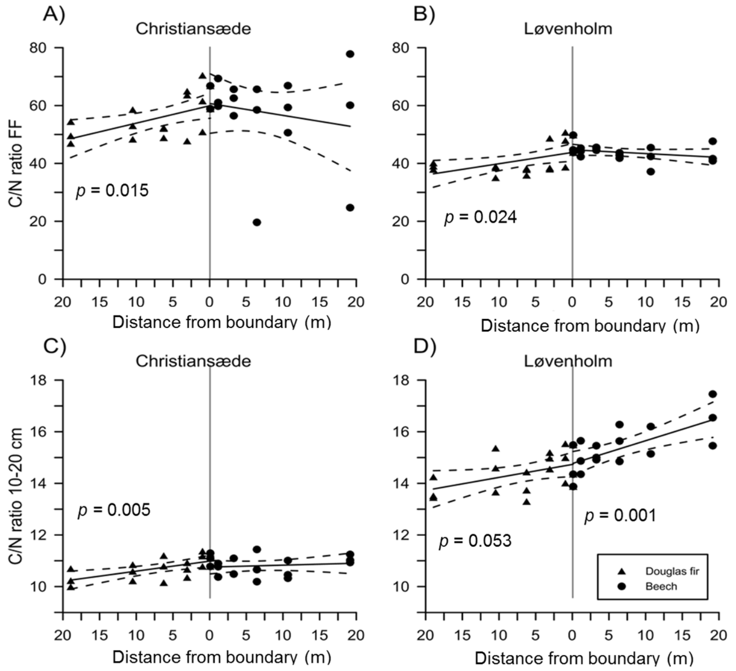

Figure 4. Response of the C/N ratio in the forest floor (**A** and **B**) and 10–20 cm layer (**C** and **D**) along transects from pure Douglas-fir and beech stand conditions to the boundary at Christianssæde and Løvenholm.

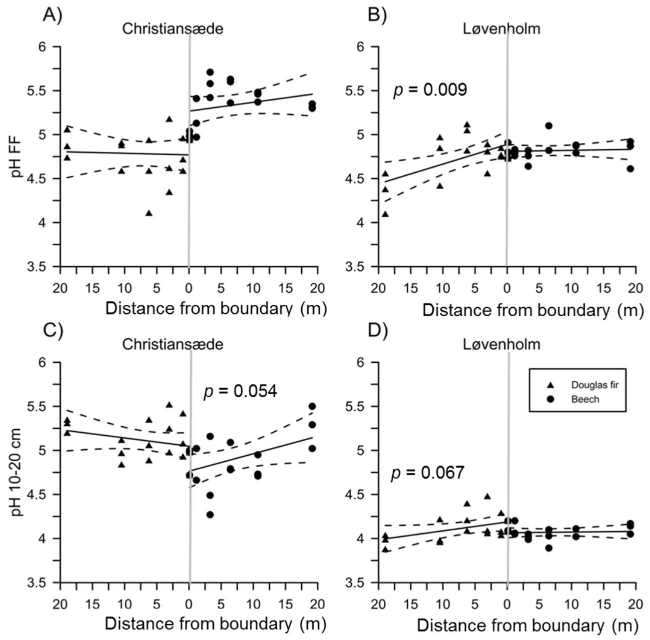

Figure 5. Response of the soil pH in the forest floor (**A** and **B**) and 10–20 cm layer (**C** and **D**) along transects from pure Douglas-fir and beech stand conditions to the boundary at Christianssæde and Løvenholm.

3. Results

3.1. Effects on Soil Properties by Sites and Tree Species

Soil C and N stocks at CHR and LØV sites were significantly different in the sampled soil layers (Table 3). Soil C and N stocks at CHR were higher than that at LØV due to higher C stocks in the mineral soil layers. In the FF, the highest C and N stocks were found at LØV. The C/N ratios were also significantly different. In the FF, the highest ratio was found at CHR whereas in the mineral soil layers, the highest ratios were found at LØV. Soil pH was significantly higher at CHR in all soil layers than that at LØV.

Table 3. Mean and standard error (SE) for the studied soil properties by site and soil layer.

Site	Soil Layer	N	C Stock ($Mg{\cdot}ha^{-1}$)		N Stock ($Mg{\cdot}ha^{-1}$)		C/N Ratio		pH	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE
CHR	FF	36	6.3 b	1.4	0.1 b	0	56.9 a	1.9	5.1 a	0.1
	0–5cm	36	20.8 a	0.7	1.4 a	0	14.7 b	0.2	4.4 a	0.1
	5–10cm	36	13.6 a	0.4	1.1 a	0	12.1 b	0.2	4.5 a	0
	10–20cm	36	22.2 a	0.4	2.1 a	0	10.8 b	0.1	5.0 a	0
	FF + 0–20cm	36	62.9 a	1.6	4.7 a	0.1	13.3 b	0.2	-	-
LØV	FF	36	11.8 a	0.7	0.3 a	0	42.5 b	0.7	4.8 b	0
	0–5cm	36	15.4 a	0.6	0.9 b	0	17.3 a	0.2	3.9 b	0
	5–10cm	36	8.2 b	0.2	0.5 b	0	15.3 a	0.3	3.9 b	0
	10–20cm	36	13.9 b	0.4	0.9 b	0	14.9 a	0.2	4.1 b	0
	FF + 0–20cm	36	49.3 b	1.3	2.7 b	0.1	18.6 a	0.2	-	-

Identical soil layers with different letters in CHR and LØV sites were significantly different for that soil property.

Soil C and N stocks under the two tree species were almost similar in all soil layers (Table 4). The only significant difference was in the FF where Douglas-fir accumulated more C and N than beech. Douglas-fir also had a significantly higher soil C/N ratio in the top mineral soil layer (0–5 cm layer). In the uppermost layers (FF and 0–5 cm), Douglas-fir had slightly lower pH than beech.

Table 4. Mean and standard error (SE) for the examined soil properties by tree species and soil layer.

Species	Soil Layer	*n*	C Stock (Mg·ha^{-1})		N Stock (Mg·ha^{-1})		C/N Ratio		pH	
			Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Beech	FF	30	7.4 b	0.8	0.2 b	0	50.3 a	2.4	5.1 a	0.1
	0–5cm	30	17.6 b	0.6	1.2 a	0.1	15.4 b	0.3	4.3 a	0.1
	5–10cm	30	10.8 a	0.5	0.9 a	0.1	13.6 a	0.5	4.2 a	0
	10–20cm	30	18.0 a	0.8	1.5 a	0.1	13.1 a	0.5	4.5 b	0.1
	FF + 0–20cm	30	53.8 a	1.4	3.6 a	0.2	15.5 a	0.6	-	-
Douglas-fir	FF	30	11.3 a	1.7	0.3 a	0	47.6 a	1.7	4.7 b	0
	0–5cm	30	18.4 a	0.8	1.1 a	0.1	16.5 a	0.4	3.9 a	0
	5–10cm	30	10.3 a	0.6	0.8 a	0.1	13.4 a	0.4	4.2 a	0
	10–20cm	30	18.1 a	0.8	1.5 a	0.1	12.5 a	0.4	4.6 a	0
	FF + 0–20cm	30	58.1 a	2.3	3.7 a	0.2	16.2 a	0.5	-	-

Identical soil layers with different letters in beech and Douglas-fir stands were significantly different for that soil property.

3.2. Effects of Species Mixture Gradients on Soil Properties

Strong site and species interactions were found when the data was analyzed by a model including site, species, distance, transect and their interactions (Tables S9–S12). Site explained the largest part of the variation (>82%) in C and N stocks, and C/N ratios for all layers, and in pH in the mineral soil layers (Table 5). In the FF, the pH variation was explained almost equally by site and species. The contribution to the variation by distance and transect was minor (<10% and mostly <5%).

Table 5. Proportion of variation in soil properties explained by each explanatory variable by each soil layer. See Table S13 for the R^2 values of the models.

Soil Layers	Variables	R^2			
		C Stock	**N Stock**	**C/N Ratio**	**Soil pH**
Forest Floor	Site	87%	88%	83%	37%
	Species	8%	6%	2%	48%
	Distance	2%	4%	10%	0%
	Transect	2%	3%	5%	4%
	Site: Distance	-	-	-	6%
	Species: Distance	-	-	-	5%
0–5 cm	Site	94%	99%	84%	65%
	Species	1%	0%	9%	17%
	Distance	0%	0%	0%	3%
	Transect	3%	0%	3%	9%
	Site: Distance	2%	-	3%	-
	Species: Distance	-	-	-	6%
5–10 cm	Site	92%	99%	97%	93%
	Species	1%	0%	0%	0%
	Distance	3%	0%	1%	0%
	Transect	1%	1%	2%	6%
	Site: Distance	3%	-	-	-
10–20 cm	Site	96%	99%	96%	94%
	Species	0%	0%	2%	2%
	Distance	0%	0%	0%	0%
	Transect	1%	0%	1%	1%
	Site: Distance	3%	-	0%	2%
	Species: Distance	-	-	2%	-
FF + 0–20 cm	Site	82%	98%	98%	-
	Species	4%	0%	1%	-
	Distance	0%	0%	0%	-
	Transect	4%	1%	0%	-
	Site: Distance	9%	1%	-	-

The influences of tree species mixing, as represented by distance to the boundary, varied between sites and soil layers (Tables S1–S8). Because the site and interaction effects were so strong, we performed further analyses of the mixture effects separately for the two sites. We did this by the transect approach and looked for significant changes (regression slopes) in soil properties along transects from the monocultures to the boundaries (Figures 3–5). No synergistic mixture effects could be identified for C and N stocks and pH whereas several single-sided effects of mainly beech on Douglas-fir were found. Stocks of both C and N at LØV were significantly higher in the pure Douglas-fir end of the transects than in the boundary where the influence of beech increased). This influence of beech mainly occurred in the forest floor and 10–20 cm layer of the mineral soil (Table S2). At CHR, this effect was absent. The beech effect on Douglas-fir was stronger for N than for C, resulting in decreasing C/N ratios from the boundary to the pure Douglas-fir end of the transects. This clear pattern was found at both sites and for both forest floor and mineral soil layers (Figure 4). In the mineral soil, but only at LØV, Douglas-fir influenced the C/N ratio in beech similarly (Figure 4D), i.e., there was a double-sided but additive effect of admixture. A significant positive influence of beech on pH in Douglas-fir was also seen at LØV, both in the forest floor and in the mineral soil.

4. Discussion

4.1. Tree Species Effects

Douglas-fir and beech are known to influence the mineral soil relatively similarly in terms of C and N stocks, the C/N ratio and pH, but forest floor C and N stocks and C/N ratios tend to be higher and pH lower in Douglas-fir than beech [17,18,40,41]. Our results were in line with this general experience and thus only partly supported our hypothesis that beech and Douglas-fir would not differ in soil properties. Some modest exceptions were observed, e.g., higher C and N stocks in forest floors and higher acidity in the forest floor and top 5 cm of the mineral soil in Douglas-fir. The tree species effect was limited compared to the effect of site, albeit climatic conditions were quite similar and soils at the two sites were both developed from till deposits of similar age. The LØV site with a more coarse-textured parent material had generally lower pH and higher C/N ratios, and higher C and N stocks in the forest floor but lower C and N stocks in the mineral soil. These characteristics suggest more restricted availability of N and base cations at LØV than at CHR. However, a modest but consistent soil property signal of the two tree species was observed approximately 50 years after the establishment of the common garden experiment. We found no site-specific effects of the two tree species, in line with previous studies of beech and Douglas-fir forest floor C stocks across seven common garden experimental sites including CHR and LØV [18]. Cremer et al. [40] also reported that Douglas-fir had consistently higher soil C and N stocks across three German sites, but the two species had similar forest floor C/N ratios in soils developed from nutrient-rich parent material, whereas C/N ratios were higher in Douglas-fir than in beech and similar to spruce in a soil formed from sandstone.

The limited but consistent effects of the two tree species mainly on the forest floor and top mineral soil suggests an influence on soils via litterfall. Litterfall amounts are quite similar among different tree species within the same climatic conditions [26,42], and foliar litter mass and nutrient concentrations did not differ significantly between beech and Douglas-fir in a Danish common garden experiment [42]. In a Polish common garden experiment, Hobbie et al. [43] reported a slightly higher C/N ratio and lower base cation contents in incubated foliar litter of Douglas-fir compared to beech, but similarity in carbon fractions including lignin, and beech litter decomposed slower than that of Douglas-fir. Higher base cation concentrations in beech litterfall may explain our observation of higher pH in the forest floor and topsoil under beech, and the higher C and N stocks in the forest floor under Douglas-fir would be best explained by the slower turnover of Douglas-fir forest floors in accordance with observations by Reich et al. [41]. Based on our observations and related literature, we conclude that the two tree species are relatively comparable in terms of litter quality and influences on soils, and as such more similar than the trends reported for Norway spruce compared to other common European broadleaves [26,44].

4.2. Effects of Beech and Douglas-Fir Mixtures

Previous studies of tree species mixtures have often focused on mixing non-N fixing and N-fixing tree species with an aim to improve the overall nutrition and growth rates [3]. Recent studies of soil properties in mixtures or along species diversity gradients have also tended to mix tree species with a relatively wide range in tree species traits such as litter nutrient and lignin concentration [24,45]. It is therefore interesting to test whether beech and Douglas-fir, given their relatively similar footprints on the soil in monocultures, would lead to synergistic (non-additive) effects on key soil properties in mixtures. The transboundary approach demonstrated single-sided effects, i.e., mainly an effect of adjacent beech on Douglas-fir, but no synergistic effects, i.e., a double-sided unidirectional effect toward the stand boundary (Figure 2). Beech reduced the C and N stocks of the soil at the nutrient-poor site, caused an increase in the C/N ratio in the forest floor and mineral soil at both sites, and reduced the acidifying effect of Douglas-fir at the nutrient-poor site. These results do not support our hypothesis that a beech and Douglas-fir mixture would result in synergistic mixture effects on soil properties and did not support the hypothesis that mixture effects would be consistent across sites and soil layers. The presence of non-additive effects would have required increasing (or decreasing) values of soil properties toward the boundary while we rather observed a mediating effect of beech on soil properties in the Douglas-fir stand. This result is, to some extent, in line with reports from mixtures of Douglas-fir and beech in Germany, but here the single-sided effect of the species mixture occurred for beech because forest floor and mineral soil C stocks were higher in beech–Douglas-fir mixtures than in beech monocultures [40]. Similar to our site-specific results, Cremer et al. [40] found no general effect of beech–Douglas-fir mixtures on C and N stocks in the entire sampled profile across site and soil types.

The single-sided effect of beech on Douglas-fir, resulting in reduced C and N stocks, higher forest floor C/N ratios and higher soil pH at LØV, can be explained by increased rates of decomposition of foliar litter with increasing influence of beech litterfall in the Douglas fir stand. Faster decomposition of slightly more base cation rich litter leads to less acidification in the forest floor and topsoil [46] and faster turnover of forest floor material is also indicated by the higher C/N ratios as such forest floors are mainly composed of recent foliar litter [26,47]. There were larger responses to species mixing at the more nutrient-poor and sandy site. Clayey and loamy soils are known to buffer influences by tree species more strongly than sandy soils [48] and this would explain why we see more effects of beech on Douglas-fir at LØV but not at the more clay-rich and well-buffered CHR site, even in a perspective of 50 years.

We attribute the influence of species mixture to the dynamics of litterfall based on the fact that soil changes were mainly observed in the forest floor and top mineral soil. Litterfall amounts of beech and Douglas-fir were probably not symmetrically distributed around the boundary. In their review of German studies in beech and Norway spruce forests, Rothe and Binkley [3] reported that 20%–70% of beech leaf litter was deposited under spruce canopies while only 5%–20% of spruce needle litter was deposited under beech canopies. A similar pattern was reported by Lavery et al. [16] in a transboundary study of alder (*Alnus rubra* (Bong.) Carr.) and various conifers in British Columbia. Alder leaf litter migrated 8–18 m into the conifer stands whereas no conifer litter migrated more than 5 m into the alder stands. In the more harsh Danish wind climate, these central European and Canadian results may not full apply, but we noted migration of beech foliar litter 5–10 m into Douglas-fir at both sites whereas Douglas-fir litter migrated no more than 3 m into beech at CHR and a bit further at LØV. Because of the likely litterfall-driven effects and the species-specific patterns in leaf litter distribution, we suggest that the wider migration of beech leaf litter into the Douglas-fir stand is an important mechanism behind the single-sided effect of beech on Douglas-fir soil properties.

A few other characteristic patterns emerged from the transboundary approach. Forest floor pH at CHR (Figure 5A) showed no mixture effect at all but only a general tree species difference (Figure 2). The gradual decrease in the C/N ratio in 0–20 cm at LØV from the pure beech stand via the boundary to the pure Douglas-fir stand (Figure 4D) was an example of strictly additive mixture effects.

The lack of synergistic effects on soil properties may be attributed to the relatively similar tree species traits, i.e., litter quality or rooting depth, that prevented any larger niche differentiation and complementarity. Dawud et al. [22] reported higher SOC contents in mixed stands compared to respective monocultures in Polish forests that were related to deeper rooting and possible higher C inputs to the mineral soil (20–40 cm) because of more efficient exploitation of the soil by roots in mixed stands. However, this occurred mainly in stands of three or more species where the likelihood of complementarity was higher. Our sampling was probably too shallow to sufficiently assess differences in SOC stocks by differential rooting patterns, but when grown in mixtures with conifers, beech was reported to develop denser fine roots at 5–20 cm depth [49] and had a tendency of differentiating its below-ground niche, for example, to take up water from deeper layers [50]. Tree species with higher functional trait complementarity would probably have been more likely to show synergistic species mixture effects on soil properties [22,23]. A large-scale study along a well-defined environmental gradient would be required to disentangle the context-dependent beech–Douglas-fir mixture effects on soils.

4.3. The Transboundary Approach

The transboundary approach proved useful to study various forms of species mixture effects (Figure 2). This approach has been used only on a few previous occasions to evaluate the effects on soil C and N [15,16] and N cycling indices [15,16] of mixing N-fixing and non-N fixing tree species. Mixture effects were observed to various degrees around the boundary between these tree species with widely different N cycling traits, but they were also site-specific as in our case [15,16]. These previous studies focused on ameliorating the effects of one N-fixing tree species on the growth or N cycling in the neighboring stand rather than testing the hypothesis of whether synergistic or purely additive effects would occur in tree species mixtures. The interest in tree species diversity as a possible driver of ecosystem functions and services [1] has increased the demand for new long-term common garden experiments with tree species mixtures and several have been established in recent years [51–53]. However, these young common garden experiments have limited current value for studies of tree species mixtures or diversity effects on soils given the slow rate of change in soil properties. Exploratory platforms in mature forests have been carefully selected to overcome this issue [54] but even careful selection requires accounting for site factors to avoid mixed-species effects from being confounded with, e.g., soil type. Based on the current study, we suggest that existing mature common garden experiments be used to test tree species mixture effects until new common garden species diversity reaches greater maturity. The advantages are the longer-term perspective of such existing experiments and the controlled site and soil conditions and well-defined gradients in species dominance, while the drawbacks are the limited combinations of species, particularly for mixtures of more than two species, as defined by existing experimental designs.

5. Conclusions

In this transboundary investigation of the possible effects of mixed forests of Douglas-fir and European beech, we detected interactions in topsoil properties between the adjacent stands of the two tree species belonging to different functional groups. However, mixture responses in C and N stocks, the C/N ratio and pH were site- and soil layer-specific and were mainly single-sided. The neighboring beech stand affected Douglas-fir, and there was no evidence of synergistic effects toward the boundary between the two tree species. Beech reduced the soil C and N stocks in Douglas-fir at the nutrient-poor site and caused an increase in the C/N ratio in the forest floor and mineral soil at both sites and reduced the acidifying effect of Douglas-fir at the nutrient-poor site. These results do not support our hypotheses that a beech and Douglas-fir mixture would result in synergistic effects on soil properties and mixture effects would be consistent across sites and soil layers. The lack of synergistic effects may be attributed to the relatively similar traits of the two tree species, i.e., litter quality or rooting depth that prevented any larger niche differentiation and complementarity.

The results indicate that the effects of Douglas-fir and beech mixtures on topsoils are context dependent. More studies will be required to determine whether an effect of admixture will occur in subsoils and to ultimately recommend in which site types beech and Douglas-fir mixtures would be conducive to higher soil C and N stocks or higher pH and lower C/N ratios than the respective monocultures. The transect approach within a mature single species common garden experiment proved useful as a platform to test tree species interactions, and we suggest that this approach be explored in soil studies until dedicated mixed species common garden experiments reach greater maturity.

Supplementary Materials: The followings are available online at www.mdpi.com/1999-4907/8/4/95/s1, Table S1: Within site effects on soil C stock in CHR by soil layers; Table S2: Within site effects on soil C stock in LØV by soil layers; Table S3: Within site effects on soil N stock in CHR by soil layers; Table S4: Within site effects on soil N stock in LØV by soil layers; Table S5: Within site effects on soil C/N ratio in CHR by soil layers; Table S6: Within site effects on soil C/N ratio in LØV by soil layers; Table S7: Within site effects on soil pH in CHR by soil layers; Table S8: Within site effects on soil pH in LØV by soil layers; Table S9: Across site effects on soil C stock by soil layers; Table S10: Across site effects on soil N stock by soil layers; Table S11: Across site effects on soil C/N ratio by soil layers; Table S12: Across site effects on soil pH by soil layers; Table S13: R-squared values of across site models by soil layers and soil properties.

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