Changes in tree layer and altitudinal distribution of herbaceous species in temperate old-growth forests over 30 years

Pavel Unar,
David Janík,
Dušan Adam,
Jan Holík

Temperate forest ecosystems, including old-growth fragments, are subject to increasing pressures, both from biotic and abiotic factors. Frequent disturbance events, rising mean annual temperatures and longer-lasting droughts are causing changes in tree species composition, probably shifting the altitudinal distribution of herbaceous species as well. Our goal was to examine whether such shifts can be observed even in old-growth temperate forests, and if the changes in the species composition and spatial distribution of trees is reflected in the herbaceous layer. Our study was based on a survey of several old-growth forests from the 1970s that was repeated after 30 years. Using spatial point pattern methods and generalized linear mixed effect models, repeated measurements of mapped phytosociological relevés and detailed maps of tree positions from two survey periods allowed us to examine how the species composition of the herb layer and the spatial distribution of trees ≥ 10 cm DBH (diameter at breast height) changed over 30 years. On most of the studied sites, the total number of trees declined and the proportion of broadleaves increased between the two surveys. Analyses of tree spatial distribution showed a general shift from a regular spatial distribution in the 1970s to a clustered spatial distribution of trees in the 2000s. In the 2000s, herbaceous species showed an upwards shift in their distribution compared to the 1970s, even after accounting for the effect of changing tree spatial distributions in both survey periods. These effects could be an outcome of warmer and drier weather conditions during the past decades. Further investigation is needed to examine whether this trend is related to changes in climatic conditions.

Keywords: Herb Layer, Species Presence, Forest Structure, Altitude, Climate Conditions

Introduction

In the past decades, forests in Central Europe have come under increasing pressure, for example from repeated outbreaks of insect pests (Müller et al. 2008), fungal pathogens (Kowalski 2006), rising mean annual temperatures and longer-lasting drought periods (Bréda et al. 2006). Forest

☐ The Silva Tarouca Research Institute for Landscape and Ornamental Gardening, Department of Forest Ecology, Lidická 25/27, Brno (Czech Republic)

@ Pavel Unar (pavel.unar@vukoz.cz)

structure is more and more being changed by disturbances, which, among other things, change tree species composition (Seidl et al. 2017). Moreover, the effects of climate change on the mortality and growth of trees has gradually led to shifts in tree species dominance (Anderegg et al. 2013). This is exemplified by the trend of beech expansion in central European old-growth forests during the past decades (Jaloviar et al. 2017). Its share is growing both at the expense of long-term receding fir and at the expense of bark beetle-weakened spruce (Danek & Vrška 2019).

Changes in the species composition and spatial distribution of trees are reflected in the cover and species composition of the herb layer (Helm et al. 2017, Nagel et al. 2019). In old-growth forests, higher light availability in canopy gaps is often a key driver of changes in the herb layer (Degen et al. 2005). The diversity of the herb layer usually increases after canopy gaps are formed, but is gradually reduced with an increasing cover of woody regeneration (Muscolo et al. 2014). However, the structure and species composition of the herb layer can also reflect the legacy effects of long-term tree canopy dynamics (Graf et al.

2019). Rising mean annual temperatures and more common drought periods observed during recent decades could also have impacted the herb layer in old-growth forests. Further research focused on changes in the species composition and distribution of herbaceous species would help clarify the effect of both forest dynamics and climate.

Shifts in the distribution of herbaceous species along altitudinal or latitudinal gradients (Walther et al. 2005, Lenoir et al. 2008) are usually interpreted as an outcome of rising temperatures (Helm et al. 2017). Many studies reporting shifts in the distribution of herbaceous species have focused on alpine vegetation (Stanisci et al. 2016), but the consequences of rising temperatures can also be noticed in forest ecosystems at lower altitudes. In relation to rising temperatures, changes in tree recruitment, decreasing relative cover of herbaceous species, and also increasing compositional similarity to communities in warmer regions have been observed (Harrison et al. 2010). Nevertheless, the question of whether there is a shift in the distribution of forest herbaceous species along the altitudinal gradient with respect to the

Tab. 1 - Studied sites. (N): number of relevés.

Locality	Area (ha)	Latitude N	Longitude E	Altitude (m a.s.l.)	N	First survey	Repeated survey
Boubín	46.6	48° 58′ 43″	13° 48′ 43″	925-1107	23	1972	2006
Milešice	8.9	48° 59′ 05″	13° 50′ 21″	1070-1125	2	1972	2011
Stožec	16.2	48° 52′ 50″	13° 50′ 06″	750-900	16	1974	2013
Žákova hora	17.5	49° 39′ 20″	15° 59′ 39″	752-800	22	1974	2011
Žofín	74.2	48° 39′ 58″	14° 42′ 28″	730-837	42	1975	2008

spatial distribution of trees has not yet been sufficiently answered. We examined changes in the composition and spatial distribution of species in Central European old-growth forests over the past 30 years. The research questions were as follows: (i) did the composition and spatial distribution of trees change over the period of 30 years? (ii) Did herbaceous species shift upwards along the altitudinal gradient during the studied period? (iii) Could an upward shift in altitude be observed for herbaceous species, after controlling for co-occurring changes in the spatial distribution of the tree layer?

Methods

Data collection

We used data from five natural forest sites in the Czech Republic belonging to the Hercynian and Bohemian-Moravian mountain region. Specifically, these included Boubín, Milešice, and Stožec in the Šumava mountains, Žofín in the Novohradské hory mountains and Žákova hora in the Českomoravská vrchovina highlands (Tab. 1).

All the studied sites have been unmanaged for decades. Cambisols are the predominant soils at these sites, with fragments of hydromorphic soils in Boubín and Žofín. Boubín, Stožec, Milešice, and Žofín are mostly north-east to north-west oriented with slopes of 5-25°. Žákova hora is south-west oriented with slopes up to 15°. Except for Milešice, beech dominates the studied sites with occurrences of either spruce or fir. Milešice represents a mountain spruce forest with rare occurrences of fir in the main canopy layer and beech in the upper understorey. The sites are fenced, except for Stožec and Žákova hora. The mean annual precipitation is 740 mm and the mean annual temperature 6.1 °C in Žákova hora (Vrška et al. 2002). At the other sites, mean annual precipitation is about 800 mm and the mean annual temperature about 4-5 °C (Vrška et al. 2012). Mean annual temperature and precipitation in decades during the period of the survey, processed on the basis of data from Czech Hydrometeorological Institute (https://www.chmi.cz), are shown in Tab. 2.

All standing and lying tree stems ≥ 10 cm DBH were repeatedly mapped and identified to species level (Tab. 3 and Tab. S1-S5 in Supplementary material). The spatial distribution of trees in 50 × 50 m plots was used for spatial point pattern analyses, with each plot concentric with a phytosociological relevé. The first large-scale measurements, including the establishment of phytosociological relevés, date back to the 1970s (Pruša 1985). The distribution of relevés respected the site-specific level of habitat heterogeneity. The relevés were circular with a diameter of 25 m. Detailed maps of standing and lying trees and phytosociological relevés were updated at the turn of the millennium (Vrška et al. 2012, Janík et al. 2016a, 2016b). The centres of phytosociological relevés were relocated in stem position maps. Based on the position of standing and lying tree stems, we could repeatedly localise the centres of phytosociological relevés with a precision of 1-2 m and thus minimize relocation error (Verheyen et al. 2018). In total, we used 105 plots, of which 42 were in Žofín, 23 in Boubín, 16 in Stožec, 22 in Žákova hora, and two in Milešice.

Data analysis

A nonparametric Wilcoxon paired test for dependent samples was used to compare the number of coniferous and broadleaved trees in the 50 × 50 m plots between the initial and repeated surveys. The difference in the proportion of coniferous and broadleaved trees between surveys was assessed with Fisher's exact test (Tab. 4). We used the pair correlation function to describe the changes in spatial patterns of trees over 30 years (Stoyan & Stoyan 1994). We compared the results of spatial analyses from the first 1970s and repeated 2000s surveys to examine whether the spatial distribution of trees had the potential to drive a possible shift in the distribution of herbaceous species along the altitudinal gradient. This function is defined by Stoyan & Penttinen (2000) as follows: "consider two infinitesimally small discs of areas dx and dy at distance r. Let $p(r)$ denote the probability that each disc contains a point of the process". Then (eqn. 1):

$$p(r) = \lambda 2 g(r) dx dy \qquad (1)$$

where λ is the density of trees. According to Law et al. (2009), the function $g(r)$ is a normalised measure of how, on average, a plant perceives the density of other plants as it "looks out" over increasing distances r into the community in which it lives. The pair correlation function has a non-cumulative character; it uses annuli as distance classes. Under the assumption of a homogenous Poisson process, $g(r) = 1$. Values of $g(r)$ larger than one indicate clustering, while values smaller than one indicate regularity (Illian et al. 2008). The pair correlation function $g(r)$ was estimated for each plot (n = 105) within the interval 0.5-4.0 m in steps of 0.5 m. The nonparametric bias-corrected and accelerated (BC_a) confidence intervals (95%) based on 1000 bootstrap replications (Efron & Tibshirani 1993) were constructed for these mean $g(r)$ values. The BC_a limits were given by the percentiles of the bootstrap distribution, which were related to acceleration and bias correction (Efron & Tibshirani 1993). To perform spatial analysis, the minimum tree density was 10 individuals per plot (Aldrich et al. 2003). Spatial analyses were conducted in R (R Core Team 2020), using

Tab. 2 - Mean annual temperature and precipitation in decades during the period of survey. Mean values for whole Czech Republic (CZ) and for regions where surveyed forests are located (South Bohemia, Czech-Moravian highlands) are shown. Processed on the basis of data from the Czech Hydrometeorological Institute (https://www.chmi.cz).

Parameter	Locality	Period				
		1970-1979	1980-1989	1990-1999	2000-2009	2010-2019
Mean temperature (°C)	CZ	7.36	7.33	7.97	8.39	8.71
	South Bohemia	6.84	6.91	7.39	7.88	8.26
	Highlands	6.94	6.87	7.46	7.97	8.53
Annual precipitation (mm)	CZ	655.8	668.9	659.6	703.9	657.5
	South Bohemia	655.8	653.6	644.4	743.2	672.3
	Highlands	677.5	650.7	623.3	702.6	633.6

Tab. 3 - Number and volume of living trees and volume of deadwood in studied localities, average cover of herb layer and number of species of herb layer recorded in relevés in the survey periods. More detail information about number and volume of living trees is provided in Supplementary material (Tab. S1-S5).

Layer	DBH Class	Parameter	Boubín		Milešice		Stožec		Žofín		Žákova hora	
			1972	2000	1972	2011	1974	2013	1975	2008	1974	2011
Living trees	≥ 10 cm	No. all living trees ha^{-1}	314.7	209.9	257.0	333.1	160.0	149.7	233.2	218.5	254.4	308.1
		No. deciduous trees ha^{-1}	121.0	107.6	85.9	72.1	96.7	80.1	146.5	156.8	222.9	286.9
		No. coniferous trees ha^{-1}	193.6	102.3	171.1	260.9	63.3	69.6	86.7	61.7	31.6	21.2
		Total volume (m^3 ha^{-1})	*547.0*	*674.1*	*330.3*	*632.8*	*671.6*	*648.1*	*622.6*	*539.6*	*375.5*	*666.6*
	10-39 cm	No. all living trees ha^{-1}	236.0	107.6	205.3	214.7	58.1	72.5	145.6	152.1	200.6	208.6
		No. deciduous trees ha^{-1}	96.8	60.3	72.7	30.5	26.0	29.5	110.5	117.6	186.4	202.4
		No. coniferous trees ha^{-1}	139.2	47.3	132.6	184.2	32.1	43.1	35.1	34.5	14.1	6.2
		Total volume (m^3 ha^{-1})	*134.5*	*72.4*	*132.9*	*123.5*	*56.0*	*33.2*	*89.0*	*76.4*	*106.1*	*121.6*
	40-79 cm	No. all living trees ha^{-1}	72.0	89.4	50.0	111.3	92.7	56.0	77.2	53.3	50.3	89.9
		No. deciduous trees ha^{-1}	24.0	45.9	13.2	41.1	65.9	35.6	32.7	33.0	34.1	78.2
		No. coniferous trees ha^{-1}	47.9	43.5	36.8	70.2	26.8	20.4	44.4	20.3	16.2	11.6
		Total volume (m^3 ha^{-1})	*333.8*	*456.1*	*183.4*	*447.7*	*496.2*	*327.0*	*400.1*	*294.9*	*227.2*	*430.3*
	≥ 80 cm	No. all living trees ha^{-1}	6.6	12.8	1.7	7.1	9.3	21.2	10.4	13.1	3.6	9.6
		No. deciduous trees ha^{-1}	0.2	1.4	0.0	0.6	4.8	15.1	3.3	6.2	2.3	6.2
		No. coniferous trees ha^{-1}	6.5	11.5	1.7	6.5	4.4	6.1	7.1	6.9	1.2	3.4
		Total volume (m^3 ha^{-1})	*78.7*	*145.5*	*13.9*	*61.6*	*119.3*	*287.9*	*133.4*	*168.4*	*86.4*	*192.0*
Deadwood	-	No. of deadwood (pcs ha^{-1})	111.6	153.9	40.5	126.9	14.7	86.0	32.2	122.4	42.0	68.2
		Volume of deadwood (m^3 ha^{-1})	147.8	364.6	75.9	171.7	51.0	183.3	111.9	355.1	101.2	149.6
Herb layer	-	Average cover (%)	66	53	70	10	77	65	76	51	66	28
		No. of recorded species	48	64	20	15	34	70	70	97	42	47

the library "spatstat" (Baddeley & Turner 2005). The library "bootstrap" (Efron & Tibshirani 1993) was used for the construction of BCa confidence intervals.

We applied two generalized linear mixed models with a binomial error distribution and logit link function (Bolker et al. 2009) to investigate the effects of altitude, survey period, spatial distribution of trees and the interaction of altitude and survey period on the probability of species presence. In the case of the herb layer, presence-absence data were used for analyses. Our main focus was on the interaction of altitude and survey period, because its positive/negative effect indicates that species tend to occur at a higher/lower altitude in the 2000s compared to the 1970s. In the first model, phytosociological relevé, site and species were used as random intercepts to account for variation in the baseline probability of species presence among relevés, sites and species (random intercepts model). We did not include the Milešice site in these analyses due to the low number of plots found at this site. The probability of presence p_{jks} of species j in relevé k and site s was modelled as follows (eqn. 2):

$$\log\left(\frac{p_{jks}}{1-p_{jks}}\right) = \beta_0 + \beta_1 A_{jks} + \beta_2 P_{jks} + \beta_3 \left(A_{jks} \times P_{jks}\right) + \beta_4 T_{jks} + \mu_j + \mu_k + \mu_s \quad (2)$$

where β_0 is the global intercept, β_1, β_2, β_3 and β_4 the effect of altitude (A), survey period (P), the interaction of altitude and survey period (A×P), and spatial distribution of trees (T), respectively. Parameters μ_j, μ_k and μ_s represent the random intercepts of species, relevé, and site, respectively. Because the effects of altitude, survey period and their interaction were not allowed to vary among species, this model was used to examine the mean response of any species in the community to these variables (community-wide response). The model represented a baseline against which we compared the second more complex model.

In the second model, we added species-specific random slopes to β_1, β_2 and β_3, which, in contrast to the previous model, allowed each species to vary in its response to altitude, survey period, and their interaction (random slopes model). It was specified as follows (eqn. 3):

$$\log\left(\frac{p_{jks}}{1-p_{jks}}\right) = \beta_0 + \beta_{1j} A_{jks} + \beta_{2j} P_{jks} + \beta_{3j} \left(A_{jks} \times P_{jks}\right) + \beta_4 T_{jks} + \mu_j + \mu_k + \mu_s \quad (3)$$

where β_1- β_3 were allowed to vary among species.

The performance of these two model types (random intercepts *vs.* random slopes models) was compared with a likelihood ratio test (Bolker et al. 2009). If the p-value of the likelihood ratio test was less than 0.05, we considered species to vary

Tab. 4 - Changes in tree layer between surveyed periods. The total number of trees ≥ 10 cm DBH in 50 × 50 m plots recorded at all sites in two survey periods. The proportion of coniferous (con$_{70}$, con$_{00}$) and broadleaved (leaf$_{70}$, leaf$_{00}$) trees and the results of Fisher's exact test comparing the proportion of coniferous and broadleaved between the first and repeated surveys are given.

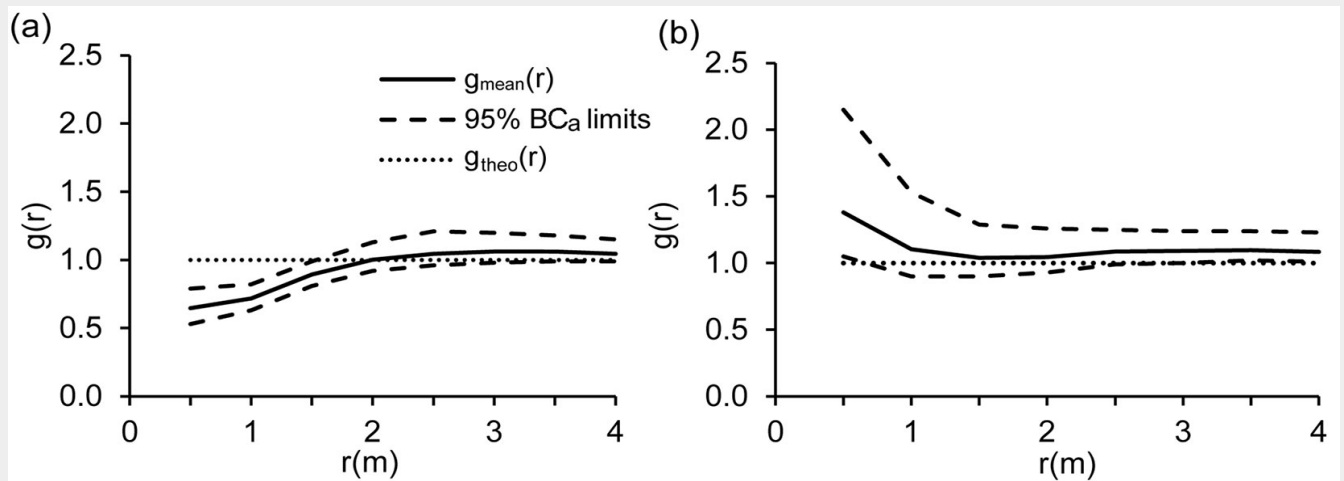| Parameter	Boubín	Milešice	Stožec	Žákova hora	Žofín
No. of trees 1970	1237	229	637	1462	1758
No. of trees 2000	785	238	548	1458	1529
con$_{70}$ (%)	62	61	41	10	23
con$_{00}$ (%)	48	69	41	6	9
leaf$_{70}$ (%)	38	39	59	90	77
leaf$_{00}$ (%)	52	31	59	94	91
Fisher's P value	*<0.0002*	*ns*	*ns*	*< 0.001*	*<0.0001*

Fig. 1 - Comparison of tree spatial patterns based on the pair correlation function g(r) in the 1970s (a) and the 2000s (b). Solid lines [$g_{mean}(r)$] show the mean values from 105 plots. Dashed lines (95% BC$_a$ limits) show the 95% nonparametric bias-corrected and accelerated confidence intervals based on 1000 bootstrap replications for the mean values of $g_{mean}(r)$. Dotted lines [$g_{theo}(r)$] show the theoretical value of complete spatial randomness. If the value of the lower 95% BC$_a$ limit is larger than $g_{theo}(r)$ then the trees show clustering at the respective distances. If the value of the upper 95% BC$_a$ limit is smaller than $g_{theo}(r)$ then the trees show a regular distribution at the respective distances. The variable "r(m)" refers to distance.

significantly in response to altitude, survey period, and their interaction. With a special focus on the interaction, we evaluated the average species-specific response to the interaction (average β_{3j}) and species-specific random slopes associated with the interaction (individual β_{3j}). Coefficients above/below zero indicate that species tended to occur at higher/lower altitudes in the 2000s compared to the 1970s.

In both models, we included 28 herbaceous species which were common to all sites: *Actaea spicata, Athyrium filix-femina, Avenella flexuosa, Calamagrostis villosa, Circaea alpina, Deschampsia cespitosa, Dryopteris carthusiana, Dryopteris filix-mas, Equisetum sylvaticum, Galeobdolon montanum, Galium odoratum, Geranium robertianum, Gymnocarpium dryopteris, Homogyne alpina, Impatiens noli-tangere, Milium effusum, Oxalis acetosella, Paris quadrifolia, Petasites albus, Phegopteris connectilis, Prenanthes purpurea, Rubus idaeus, Sanicula europaea, Senecio ovatus, Stellaria nemorum, Urtica* *dioica, Vaccinium myrtillus, Viola reichenbachiana*. Owing to variation in altitude among sites, we standardized this variable within sites by subtracting the site-specific mean and dividing by one standard deviation. The spatial distribution of trees on the 50 × 50 m plots associated with each relevé was represented by the value of the pair correlation function (Baddeley et al. 2015). It can be considered a proxy for the relative level of tree clustering in our models, with lower and higher values indicating relatively weaker and stronger tree clustering, respectively. Because the pair correlation functions indicated non-random spatial distribution of trees up to a distance of 1 m in survey periods, we separately used the values of the pair correlation function estimated at distances of 0.5 and 1 m in preliminary models with random intercepts. We compared the models with different distances and selected a distance of 0.5 m showing the lowest Akaike's Information Criterion for the final models (Burn-

ham & Anderson 2002). Generalized linear mixed models were fitted in R v. 4.0.2 (R Core Team 2020) using the library "lme4" v. 1.1-23 (Bates et al. 2015).

Results

The total number of trees ≥ 10 cm DBH per plot was significantly higher in the 1970s than the 2000s, based on the results of the Wilcoxon paired test for dependent samples (P = 0.00002). This was because the total number of coniferous trees per plot was significantly lower in the 2000s than 30 years before (Wilcoxon paired test: P < 0.0001). In contrast, the total number of broadleaved trees did not vary between the survey periods. As shown by the results of the Fisher's exact test, the proportion of broadleaved and coniferous trees significantly differed in Žofín, Boubín and Žákova hora (Tab. 4). Analyses of tree spatial patterns on 105 plots showed a general shift to more clustered patterns from the 1970s to the 2000s (Fig. 1). In the 1970s, the trees
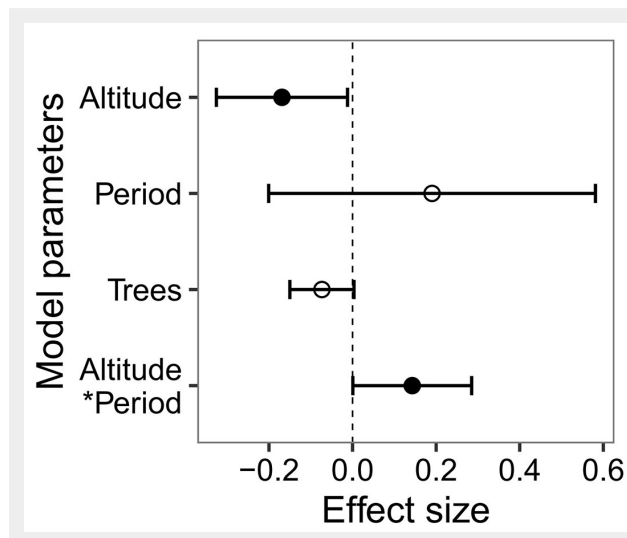

Fig. 2 - The effects of altitude, survey period (Period, P), spatial distribution of trees (Trees, T) and the interaction of altitude and survey period (Altitude × Period, A × P) on the probability of species presence based on the results of the random slopes model. Altitude was standardised within sites by subtracting the site-specific mean altitude and dividing by one site-specific standard deviation. The spatial distribution of trees is represented by relevé-specific values of the pair correlation function estimated at a distance of 0.5 m. Solid symbols denote statistically significant effects with *p* < 0.05.

largely showed a regular distribution up to a distance of 1 m and then a clustered distribution. In the 2000s, tree spatial patterns were clustered up to distances of 0.5 m. Signs of regularity completely disappeared.

By comparing the random intercepts and random slopes models with a likelihood ratio test, the random slopes model showed better fit (df = 9, χ^2 = 363.01, p < 0.001), demonstrating that species significantly varied in response to altitude, survey period and their interaction. Based on the results of this random slopes model, the probability of species presence was on average negatively related to altitude and positively related to the interaction between altitude and survey period (Fig. 2). Although non-significant, the probability of species presence was also reduced by the effect of tree clustering (Fig. 2). On average, the effect of altitude increased the probability of species presence in the 2000s compared to the 1970s (Fig. 3). This interactive effect varied among species, with almost 80% of species showing that the probability of their presence decreased more strongly with altitude in the 1970s than in the 2000s (Fig. 4).

Discussion

It is clear that the number of trees on studied plots was lower at the beginning of the new millennium than in the 1970s (Tab. 4). This could be explained by the decline of conifers owing to bark beetle outbreaks, natural forest dynamics (Král et al. 2010), or natural gap dynamics driven by the mortality and falls of individual trees or small tree groups (Kenderes et al. 2009). Possibly the intermediate-scale windstorm events of Kyrill (2007) and Emma (2008) also played a crucial role. The recruitment of tree seedlings will likely increase the number of trees with DBH ≥ 10 cm in the future, as was the case with declines of silver fir being gradually replaced by European beech in Western Carpathian natural forests (Vrška et al. 2009).

In line with our findings, the proportion of European beech has rapidly increased over approximately the same time span in many Central European old-growth forests (Vrška et al. 2009, Janík et al. 2016a, 2016b). The long-term replacement of silver fir by expanding beech has been explained by changes in forest management practices (Vrška et al. 2009), pressure from insect pests (Saniga 1999), or fungal pathogens (Szwagrzyk & Szewczyk 2001), and by the sensitivity of fir to changes in abiotic factors (Lebourgeois et al. 2010). Greater pressure on *Abies alba* from forest game can also play a role here, even though most of the studied localities are fenced. Moreover, juvenile beech trees naturally form the understory in many spruce stands (Janík et al. 2016b), which are often exposed to repeated outbreaks of bark beetles. With the exception of wet sites, the new tree generation is mostly dominated
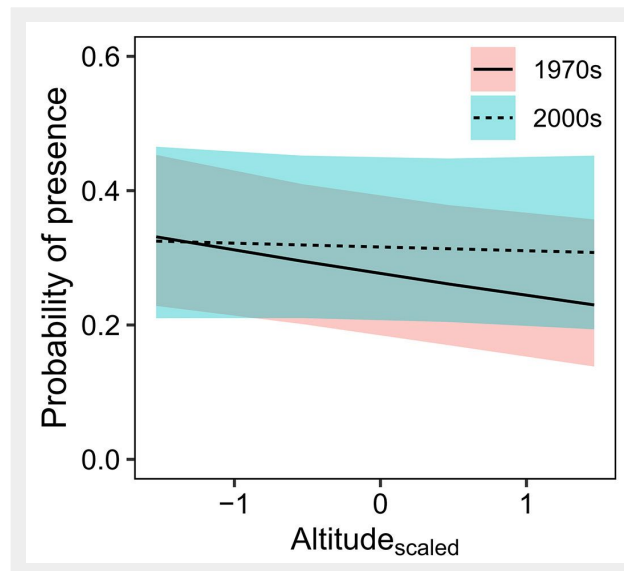
by beech in these post-disturbance areas. The replacement of tree species is thus considerably accelerated by the effects of disturbances, thereby allowing for faster adaptation of forests to changing conditions (Thom et al. 2017). Ongoing changes in the tree layer are also in line with our findings on tree spatial distributions in both survey periods. The regular spatial distribution of trees in the 1970s indicates that density-dependent processes, such as long-term competitive interactions, were important for the formation of tree spatial distributions. Conversely, we found a clustered spatial distribution of trees 30 years after the first survey in the 1970s, which can be attributed to the recruitment of new trees released by the fall of individual

trees and/or groups of trees from the aging tree generation (Getzin et al. 2008, Lutz et al. 2014).

Thinning of the tree canopy layer indicated by clustered tree distribution (Thom et al. 2020) mostly gives rise to conditions that are favourable for herbaceous species with requirements for higher light availability in the understorey. Gap dynamics and related changes in light availability are often mentioned as key drivers of changes in the understorey (Baeten et al. 2009, Naaf & Wulf 2010). On the other hand, some changes of the herb layer can be independent of changes in forest structure (Helm et al. 2017). An example could be an upward shift of herbaceous species along the altitudinal gradient as one of the outcomes
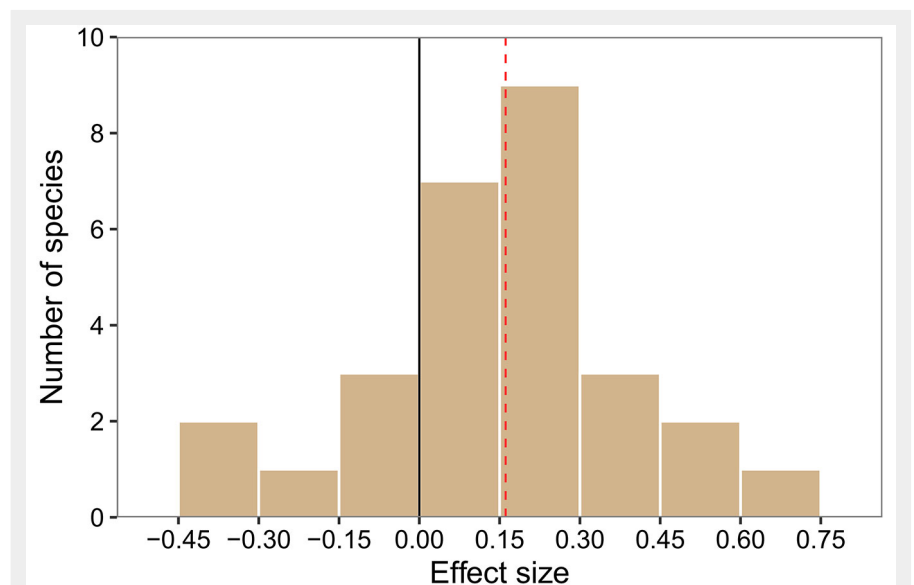

Fig. 3 - The interactive effects of altitude and survey period on the probability of species presence based on the results of the random slopes model. The predicted probabilities with 95% confidence intervals are displayed. Altitude (Altitude$_{scaled}$) was standardised within sites by subtracting the site-specific mean altitude and dividing by one site-specific standard deviation.

Fig. 4 - Distribution of interactive effects of altitude and survey period on the probability of species presence based on the results of the random slopes model. Histogram bars are based on species-specific regression coefficients (random slopes) for 28 herbaceous species. Bars to the right of the solid zero line indicate species that occur at higher altitude in the 2000s compared to the 1970s. The average species-specific response (coefficient) to the interactive effect of altitude and survey period is indicated by the red dashed line.

of rising temperatures and lower precipitation (Lenoir et al. 2008). However, it may be difficult to detect in temperate forests. The effect of changing climatic conditions can be reduced depending on the presence/absence of an upper understorey (Zellweger et al. 2020) and thermophilous plants can be dramatically suppressed due to shading by the tree canopy (De Frenne et al. 2015). Our findings show an increase in the probability of species presence at higher altitudes in the 2000s compared to the 1970s, which could indicate a shift in species distribution along the altitudinal gradient. From this perspective, it is interesting that the number of herbaceous species showing this relationship was almost 80%. Because we accounted for the effect of tree spatial distribution on species presence/absence in both survey periods, we suspect that the observed shift in the distribution of herbaceous species along the altitudinal gradient is not directly related to temporal changes in forest structure and light conditions in the understorey (Hanson & Lorimer 2007). Other biotic and environmental conditions such as light availability, precipitation and the density of juvenile woody individuals deserve further attention in future studies for the evaluation of temporal changes in the altitudinal distribution of herbaceous species.

A changing distribution of plant species along the altitudinal gradient has been associated with climate change at both regional and global scales (Walther et al. 2005). Although this cannot be directly concluded based on our findings, we demonstrate that the tree and herb layers have changed in a way that may be indicative of ongoing climate-related changes. We also show that the drivers of the distribution of herbaceous species may not be directly related to the spatial distribution of trees, and that the shifts in the distribution of herbaceous species along the altitudinal gradient can also be observed in temperate forests. Whether this is only a temporary phenomenon or rather a long-term trend needs to be examined in future studies

Conclusion

We have shown that changes in the tree layer over the 30 year period indicate a shift towards a more complex forest structure and the gradual replacement of conifers by broadleaved tree species, especially beech. We also show that herbaceous species tended, on average, to occur at higher altitudes 30 years after the initial survey in the 1970s. This relationship was observed for almost 80 % of the studied herbaceous species, even when accounting for the spatial distribution of trees in both survey periods. This was mainly related to shade-tolerant species, which are usually independent of the distribution, size of canopy openings and of early-succession stages of forest communities following disturbance events.

Acknowledgements

References

Aldrich PR, Parker GR, Ward JS, Michler CH (2003). Spatial dispersion of trees in an old-growth temperate hardwood forest over 60 years of succession. Forest Ecology and Management 180: 475-491. - doi: 10.1016/S0378-1127(02)00612-6

Anderegg WR, Kane JM, Anderegg LD (2013). Consequences of widespread tree mortality triggered by drought and temperature stress. Nature Climate Change 3: 30-36. - doi: 10.1038/nclimate1635

Baddeley A, Turner R (2005). Spatstat: an R package for analysing spatial point patterns. Journal of Statistical Software 12: 1-42. - doi: 10.18637/jss.v012.i06

Baddeley A, Rubak E, Turner R (2015). Spatial point patterns: methodology and applications with R. Chapman and Hall/CRC, Boca Raton, FL, USA, pp. 828. [online] URL: http://books.google.com/books?id=rGbmCgAAQBAJ

Bates D, Mächler M, Bolker B, Walker S (2015). fitting linear mixed-effects models using lme4. Journal of Statistical Software 67: 1-48. - doi: 10.18637/jss.v067.i01

Baeten L, Bauwens B, De Schrijver A, De Keersmaeker L, Van Calster H, Vandekerkhove K, Roelandt B, Beeckman H, Verheyen K (2009). Herb layer changes (1954-2000) related to the conversion of coppice-with-standards forest and soil acidification. Applied Vegetation Science 12: 187-197. - doi: 10.1111/j.1654-109X.2009.01013.x

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White JSS (2009). Generalized linear mixed models: a practical guide for ecology and evolution. Trends in Ecology and Evolution 24: 127-135. - doi: 10.1016/j.tree.2008.10.008

Bréda N, Huc R, Granier A, Dreyer E (2006). Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. Annals of Forest Science 63: 625-644. - doi: 10.1051/forest:2006042

Burnham K, Anderson D (2002). Model selection and multimodel inference: a practical information-theoretic approach (2nd edn). Springer, New York, USA, pp. 488.

Danek P, Vrška T (2019). Four decades of the co-existence of beech and spruce in a Central European old-growth forest. Which succeeds on what soils and why? Plant and Soil 437 (1): 257-272. - doi: 10.1007/s11104-019-03968-4

De Frenne P, Rodríguez-Sánchez F, De Schrijver AN, Coomes DA, Hermy M, Vangansbeke P, Verheyen K (2015). Light accelerates plant responses to warming. Nature Plants 1: 1-3. - doi: 10.1038/nplants.2015.110

Degen T, Devillez F, Jacquemart AL (2005). Gaps promote plant diversity in beech forests (Luzulo-Fagetum), North Vosges, France. Annals of Forest Science 62: 429-440. - doi: 10.1051/forest:2005039

Efron B, Tibshirani RJ (1993). An introduction to the bootstrap. Chapman and Hall, New York, USA, pp. 456. [online] URL: http://cindy.informatik.uni-bremen.de/cosy/teaching/CM_2011/Eval3/pe_efron_93.pdf

Getzin S, Wiegand T, Wiegand K, He F (2008). Heterogeneity influences spatial patterns and demographics in forest stands. Journal of Ecology 96: 807-820. - doi: 10.1111/j.1365-2745.2008.01377.x

Graf W, Kleinn C, Schall P, Nauss T, Detsch F, Magdon P (2019). Analyzing the relationship between historic canopy dynamics and current plant species diversity in the herb layer of temperate forests using long-term Landsat time series. Remote Sensing of Environment 232: 111305. - doi: 10.1016/j.rse.2019.111305

Hanson JJ, Lorimer CG (2007). Forest structure and light regimes following moderate wind storms: Implications for multi-cohort management. Ecological Applications 17: 1325-1340. - doi: 10.1890/06-1067.1

Harrison S, Damschen EI, Grace JB (2010). Ecological contingency in the effects of climatic warming on forest herb communities. Proceedings of the National Academy of Sciences USA 107: 19362-19367. - doi: 10.1073/pnas.1006823107

Helm N, Essl F, Mirtl M, Dirnböck T (2017). Multiple environmental changes drive forest floor vegetation in a temperate mountain forest. Ecology and Evolution 7: 2155-2168. - doi: 10.1002/ece3.2801

Illian JB, Penttinen A, Stoyan H, Stoyan D (2008). Statistical analysis and modelling of spatial point patterns. John Wiley and Sons, Chichester, UK, pp. 560. [online] URL: http://books.google.com/books?id=_U6BER2stYsC

Jaloviar P, Saniga M, Kucbel S, Pittner J, Vencurik J, Dovciak M (2017). Seven decades of change in a European old-growth forest following a stand-replacing wind disturbance: a long-term case study. Forest Ecology and Management 399: 197-205. - doi: 10.1016/j.foreco.2017.05.036

Janík D, Adam D, Hort L, Král K, Samonil P, Unar P, Vrska T (2016a). Breaking through beech: a three-decade rise of sycamore in old-growth European forest. Forest Ecology and Management 366: 106-117. - doi: 10.1016/j.foreco.2016.02.003

Janík D, Král K, Adam D, Hort L, Samonil P, Unar P, Vrška T, McMahon S (2016b). Tree spatial patterns of Fagus sylvatica expansion over 37 years. Forest Ecology and Management 375: 134-145. - doi: 10.1016/j.foreco.2016.05.017

Kenderes K, Král K, Vrška T, Standovár T (2009). Natural gap dynamics in a Central European mixed beech-spruce-fir old-growth forest. Ecoscience 16: 39-47. - doi: 10.2980/16-1-3178

Kowalski T (2006). Chalara fraxinea sp. nov. associated with dieback of ash (Fraxinus excelsior) in Poland. Forest Pathology 36: 264-270. - doi: 10.1111/j.1439-0329.2006.00453.x

Král K, Vrška T, Hort L, Adam D, Samonil P (2010). Developmental phases in a temperate natural spruce-fir-beech forest: determination by a supervised classification method. European Journal of Forest Research 129: 339-351. - doi: 10.1007/s10342-010-0361-8

Law R, Illian J, Burslem DFR, Gratzer G, Gunatilleke CVS, Gunatilleke IAUN (2009). Ecological

information from spatial patterns of plants: insights from point process theory. Journal of Ecology 97: 616-628. - doi: 10.1111/j.1365-2745.2009.01510.x

Lebourgeois F, Rathgeber CB, Ulrich E (2010). Sensitivity of French temperate coniferous forests to climate variability and extreme events (*Abies alba, Picea abies* and *Pinus sylvestris*). Journal of Vegetation Science 21: 364-376. - doi: 10.1111/j.1654-1103.2009.01148.x

Lenoir J, Gégout JC, Marquet PA, De Ruffray P, Brisse H (2008). A significant upward shift in plant species optimum elevation during the 20th century. Science 320: 1768-1771. - doi: 10.1126/science.1156831

Lutz JA, Larson AJ, Furniss TJ, Donato DC, Freund JA, Swanson ME, Bible KJ, Chen J, Franklin JF (2014). Spatially nonrandom tree mortality and ingrowth maintain equilibrium pattern in an old-growth *Pseudotsuga-Tsuga* forest. Ecology 95: 2047-2054. - doi: 10.1890/14-0157.1

Müller J, Bußler H, Goßner M, Rettelbach T, Duelli P (2008). The European spruce bark beetle *Ips typographus* in a national park: from pest to keystone species. Biodiversity and Conservation 17: 2979-3001. - doi: 10.1007/s10531-008-9409-1

Muscolo A, Bagnato S, Sidari M, Mercurio R (2014). A review of the roles of forest canopy gaps. Journal of Forestry Research 25: 725-736. - doi: 10.1007/s11676-014-0521-7

Naaf T, Wulf M (2010). Habitat specialists and generalists drive homogenization and differentiation of temperate forest plant communities at the regional scale. Biological Conservation 143: 848-855. - doi: 10.1016/j.foreco.2007.04.020

Nagel TA, Iacopetti G, Javornik J, Rozman A, De Frenne P, Selvi F, Verheyen K (2019). Cascading effects of canopy mortality drive long-term changes in understorey diversity in temperate old-growth forests of Europe. Journal of Vegetation Science 30: 905-916. - doi: 10.1111/jvs.12767

Pruša E (1985). Die böhmischen und mährischen Urwälder - ihre Structur und Ökologie [The Bohemian and Moravian primeval forests - their structure and ecology]. Academia, Praha, Czech Republic, pp. 577.

R Core Team (2020). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. [online] URL: http://www.r-project.org

Saniga M (1999). Struktúra, produkčné pomery a regeneračné procesy Badinského pralesa [Structure, production conditions and regener-

ation processes of the Badinský forest]. Journal of Forest Science 45: 121-130.

Seidl R, Thom D, Kautz M, Martin-Benito D, Peltoniemi M, Vacchiano G, Wild J, Ascoli D, Petr M, Honkaniemi J, Lexer MJ, Trotsiuk V, Mairota P, Svoboda M, Fabrika M, Nagel TA, Reyer CPO (2017). Forest disturbances under climate change. Nature Climate Change 7: 395-402. - doi: 10.1038/nclimate3303

Stanisci A, Frate L, Morra Di Cella U, Pelino G, Petey M, Siniscalco C, Carranza ML (2016). Short-term signals of climate change in Italian summit vegetation: observations at two GLORIA sites. Plant Biosystems 150: 227-235. - doi: 10.1080/11263504.2014.968232

Stoyan D, Stoyan H (1994). Fractals, random shapes and point fields: methods of geometrical statistics. John Wiley and Sons, Chichester, UK, pp. 389.

Stoyan D, Penttinen A (2000). Recent applications of point process methods in forestry statistics. Statistical Science 15: 61-78. [online] URL: http://www.jstor.org/stable/2676677

Szwagrzyk J, Szewczyk J (2001). Tree mortality and effects of release from competition in an old-growth *Fagus-Abies-Picea* stand. Journal of Vegetation Science 12: 621-626. - doi: 10.2307/3236901

Thom D, Rammer W, Seidl R (2017). Disturbances catalyze the adaptation of forest ecosystems to changing climate conditions. Global Change Biology 23: 269-282. - doi: 10.1111/gcb.13506

Thom D, Sommerfeld A, Sebald J, Hagge J, Müller J, Seidl R (2020). Effects of disturbance patterns and deadwood on the microclimate in European beech forests. Agricultural and Forest Meteorology 291: 108066. - doi: 10.1016/j.agrformet.2020.108066

Verheyen K, Baány M, Chećko E, Chudomelová M, Closset-Kopp D, Czortek P, Decocq G, De Frenne P, De Keersmaeker L, Enríquez García C, Fabšičová M, Grytnes JA, Hederová L, Hédl R, Heinken T, Schei FH, Horváth S, Jaroszewicz B, Jermakowicz E, Klinerová T, Kolk J, Kopecky M, Kuras I, Lenoir J, Macek M, Máliš F, Martinessen TC, Naaf T, Papp L, Papp-Szakály A, Pech P, Petrík P, Prach J, Reczynska A, Spicher F, Standovár T, Swierkosz K, Szczesniak E, Tóth Z, Ujházy K, Ujházyová M, Vangansbeke P, Vild O, Wulf M, Baeten L (2018). Observer and relocation errors matter in resurveys of historical vegetation plots. Journal of Vegetation Science 29: 812-823. - doi: 10.1111/jvs.12673

Vrška T, Hort L, Adam D, Odehnalová P, Horal D (2002). Developmental dynamics of virgin for-

est reserves in the Czech Republic I. Ceskomoravská vrchovina Upland - Polom, Žákova hora Mt. Academia, Praha, Czech Republic, pp. 213.

Vrška T, Adam D, Hort L, Kolár T, Janik D (2009). European beech (*Fagus sylvatica* L.) and silver fir (*Abies alba* Mill.) rotation in the Carpathians: a developmental cycle or a linear trend induced by man? Forest Ecology and Management 258: 347-356. - doi: 10.1016/j.foreco.2009.03.007

Vrška T, Unar P, Hort L, Adam D, Král K, Janík D (2012). Developmental dynamics of virgin forest reserves in the Czech Republic III. Šumava Mts. and Ceský les Mts. - Diana, Stožec, Boubín virgin forest, Milešice virgin forest. Academia, Praha, Czech Republic, pp. 238. [ISBN 80-200-0908-6]

Walther GR, Beißner S, Burga CA (2005). Trends in the upward shift of alpine plants. Journal of Vegetation Science 16: 541-548. - doi: 10.1111/j.1654-1103.2005.tb02394.x

Zellweger F, De Frenne P, Lenoir J, Vangansbeke P, Verheyen K, Bernhardt-Römermann M, Baeten L, Hédl R, Berki I, Brunet J, Van Calster H, Chudomelová M, Decocq G, Dirnböck T, Durak T, Heinken T, Jaroszewicz B, Kopecky M, Máliš F, Macek M, Marek M, Naaf T, Nagel TA, Ortmann-Ajkai A, Petrik A, Pielech R, Reczynska A, Schmidt W, Standovár T, Swierkosz K, Teleki B, Vild O, Wulf M, Coomes D (2020). Forest microclimate dynamics drive plant responses to warming. Science 368: 772-775. - doi: 10.1126/science.aba6880

Supplementary Material

Tab. S1 - Number and volume of living trees in DBH classes in locality Boubín.

Tab. S2 - Number and volume of living trees in DBH classes in locality Milešice.

Tab. S3 - Number and volume of living trees in DBH classes in locality Stožec.

Tab. S4 - Number and volume of living trees in DBH classes in locality Žofín.

Tab. S5 - Number and volume of living trees in DBH classes in locality Žákova hora.

Link: Unar_3749@suppl001.pdf