Gap Structure and Regeneration in the Mixed Old-Growth Forests of National Nature Reserve Sitno, Slovakia

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Abstract: Forest management mimicking natural processes represents an approach to maintain mixed, uneven-aged stands at small spatial scales. The reliance on natural processes, especially on natural regeneration leads to the use of gap-based regeneration as a fundamental silvicultural technique. As a baseline for such management, we investigated mixed forest in unmanaged National Nature Reserve Sitno in the Western Carpathians, which harbours extraordinary diversity on a rather small scale. To quantify the impact of gaps on gap-filling processes and to assess the role they play in recently observed changes in tree species composition we established a large (2.5 ha) permanent research plot and surveyed the status of natural regeneration, forest structure, tree species composition, and disturbance regime. Our research highlights the long-term and contemporary difficulties in the establishment of Quercus petraea (Matt.) Liebl and Fagus sylvatica (L.). Based on the provided evidence, the native tree species diversity in one of the few preserved old-growth multi-species beech-oak forest remnants is not likely to persist, what could have many implications for future ecosystem functioning. Our results suggest that variation in gap size is an important factor contributing to composition of tree species composition of natural regeneration. The recent intermediate-scale disturbance pattern dominating the old-growth beech-oak forest is beneficial to canopy recruitment of species less shade-tolerant than Fagus sylvatica, as Acer pseudoplatanus (L.), Acer platanoides (L.), and Fraxinus excelsior (L.). We discuss possible factors behind observed shifts in tree species composition and limitations for application of gap dynamics to forest practice in managed beech-oak forest systems. Overall, results of this study may help to design silvicultural measures promoting mixed-species forests to deliver a range of desired ecosystem services.

Keywords: gap dynamics; regeneration; recruitment; gapmaker; disturbance; European beech; Sessile oak; temperate forests

1. Introduction

Management approaches aiming to preserve tree species diversity in forests are becoming increasingly important. Enhanced diversity stabilises productivity and promotes resilience of forest ecosystems, while diverse forests have higher resistance against various harmful agents and provide more stable wood production under a wide range of environmental conditions [1,2]. One of the key silvicultural practices utilized in such type of management is the natural regeneration of forest stands. The basis for management concepts that stress the importance of spatial partitioning of gap resources in the maintenance of tree species diversity is research on natural disturbance regimes of old-growth forests [3].
Natural disturbance regimes allow various tree species of contrasting life traits to coexist [4,5]. In Central European forests, the main disturbing factor shaping spatial structure and thus affecting the dynamics of the ecosystem is the wind [6]. Wind disturbances create openings in forest canopy, gaps, which is associated with regeneration of various tree species [7]. The process of gap dynamics includes dying of the tree individually or as a group of several trees that are continuously replaced by subsequent natural regeneration without the negative impacts on forest sustainability over the larger forest area [8]. Large gaps created by rarely occurring high severity wind disturbances favour establishment of shade-intolerant, pioneer species. For example, wind disturbance creating a gap (patch) over 0.6 ha resulted in the establishment of *Salix caprea* (L.) in old-growth fir-beech forest in western Carpathians [9]. Single wind-thrown events of moderate severity with estimated occurrence once within the tree’s life cycle [10] favour recruitment of intermediate shade-tolerant species, such as oak, in very large gaps (patches) of approximate sizes 0.1–0.4 ha [4,11]. On the other hand, frequent, small gap-scale disturbances benefit recruitment and dominance of shade-tolerant tree species, such as fir or beech [7]. In European temperate forests with *Fagus sylvatica* dominance or co-dominance, distribution of gap sizes exponentially decreases towards large-sized gaps, whereas majority of gaps (above 90%) have size bellow 0.05 ha [12–15].

Applications of gap dynamics to forestry practice is limited [7]. Traditional management systems currently applied in stands where several target tree species with contrasting light requirements coexist may cause difficulties in regeneration and recruitment of some species (Saniga 2019—personal communication). In Slovakia, release thinnings and shelterwood systems utilizing single tree or small group (gap) cuttings are the most frequently used for natural regeneration of forest stands (irrespective their species composition).

In secondary mixed beech forests, utilization of mechanisms of natural regeneration based on tree or small group shelterwood can, paradoxically, lead to decline of biodiversity [16]. Many forest stands affected in this way result in simple undifferentiated structures dominated by single species, even the natural regeneration is successful. As a matter of fact, single tree or small group cuttings best imitate the regeneration processes in natural beech dominated forests [17]. Subsequently, small-scale regeneration methods in mixed stands may hamper the regeneration of other tree species at the favour of beech—a very competitive species and so to alter the composition of forests.

The important role of intermediate and large gaps in the gap dynamics of natural mixed beech forests has been highlighted [15], which agrees with more intensive forest management suggested to increase their species diversity [18]. Findings from other European studies also point to that management of light through sufficient canopy openings in mixed stands of oak and beech is not enough to promote and explain the development of the intermediate shade-tolerant species [19–21]. Other less studied mechanisms than the size of canopy opening are likely to play an important role and influence the final composition of tree species in gap-filling processes [22,23]. The proportion of stand disturbed, frequency of gaps, their temporal dynamics, the duration of periods of release may be more influential to saplings of shade-tolerant species than gap size [22].

The mixed beech-oak old-growth temperate forests can serve as a model base for forest management that aims to maintain high tree species and structural diversity. However, natural old-growth multi-species forests with oak (easily accessible at the colline and submontane climax forests [24]) has remained sparsely distributed in eastern Central Europe [11,19,25–29]. In these forests, under current ecological conditions, *Fagus sylvatica* increases its relative importance at the expense of oak, as mortality increases of both *Fagus sylvatica* and *Quercus petraea*, but higher for *Quercus petraea* [28,30,31].

For the purpose of this study, we selected mixed forest in unmanaged National Nature Reserve Sitno (Slovakia), which harbours extraordinary diversity on a rather small spatial scale and represents one of the best-preserved forest remnants in eastern Central Europe. In the selected forest, we established large (2.5 ha) permanent research plot to survey status of natural regeneration, forest structure, tree species composition and disturbance regime.
Our aim is to test and quantify the effects of gaps and gap-size related partitioning of natural resources (gradients of light, moisture, temperature according to Busing and White [32]), density, and canopy closure of forest stand and the spatial variability of natural conditions in the investigated area on density and tree species composition of natural regeneration.

We aim to answer following groups of mutually related questions:

(i) Are there changes in disturbance regime manifested by differences in the size, distribution or origin of recently created gaps?

(ii) What is the impact of recent disturbance regime, i.e., gap size, and type of its extension on density and species composition of natural regeneration in old-growth beech-oak forest?

(iii) How much variability in abundance of tree species is explainable by the niche environmental gradient formed between gaps and forest stand and how much variability can be attributed to spatial variation of natural conditions on the monitored area?

(iv) What are the practical implications for the management, if we aim the regeneration to preserve the species and structural diversity in beech-oak associations? How to regenerate mature mixed stands with the beech presence to avoid formation of a simple undifferentiated structure dominated by single species?

2. Materials and Methods

2.1. Study Area

The study site (48°23′55″ N; 18°52′54″ E) is located in the National Nature Reserve (NNR) Sitno in the Štiavnicke Mts., which is part of the Western Carpathians (Figure 1). The reserve established in 1951 is located between 770 and 940 m asl with prevailing S and SE exposition. Mean annual precipitation sum is 907 mm. Mean annual temperature is 14 °C. Bedrock consists of volcanic rocks, andesite, and pyroxene. The predominant soil type is eutric cambisol, well aerated, with high share of rocks, and with rapid draining. The mixed beech-oak forests are naturally formed by more than eight tree species: *Acer pseudoplatanus* (L.), *Acer platanoides* (L.), *Fraxinus excelsior* (L.), *Fagus sylvatica* (L.), *Tilia cordata* (Mill.), *Ulmus scabra* (Huds.), *Carpinus betulus* (L.), and *Quercus petraea* (Matt.) Liebl.

The research was conducted on a 2.5 ha (250 × 100 m) permanent research plot (PRP) established in the reserve in 2011 (Figure 1; [33]). The location of the plot was selected in the central core zone of the reserve (800–900 m asl) and corresponds with eumesotrophic site having a moderate-rich amount of dissolved nutrients. Study area comprises two main forest associations, *Querceto-Fagetum tiliosum* and *Tilieto-Aceretum*. *Fagus* and *Quercus* naturally represent this habitat with respective proportion from 30% to 50% and from 5% to 20%, and with higher admixture of above-mentioned broadleaved tree species.

2.2. Field Measurements and Data Analysis

We recorded two types of gaps in accordance with Runkle [5]: open canopy gap and expanded gap. Gaps were defined as openings in the forest canopy >5 m² formed due to the mortality of one or more canopy trees with diameter at breast height (dbh) above 25 cm. We considered gaps with the gapmaker (fallen tree causing the canopy opening) presented on the ground. The gap was not recorded and considered to be closed, when the next generation of trees reached the height layer corresponding to one third of upper (dominant) stand height—34.1 m. The positions and crown projections of trees surrounding the canopy gap were gathered, and the gap sizes were calculated using the Field-Map® software package (IFER—Monitoring and Mapping Solutions, Ltd., Jilové u Prahy, Czech Republic).

Location and areas of natural regeneration polygons were initially mapped on the whole PRP by Field-Map (Figure 1). Subsequently, the status of natural regeneration was surveyed on 3.14 m² circular plots (i.e., 1 m in radius) established on the grid intersections (n = 216, grid of 10 × 10 m) of the whole PRP. From the 216 plots, 125 plots were placed in expanded gaps and 91 under closed forest canopy. The number of individuals of each species and their height was recorded. The regeneration was classified into two height categories, which include seedlings (≤50 cm of height) and saplings
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Our aim is to test and quantify the effects of gaps and gap-size related partitioning of natural regeneration was also examined by employing ratio calculated as

Figure 1. (a) Location of the National Nature Reserve (NNR) Sitno in Slovakia; (b) permanent research plot (PRP; 250 × 100 m) with horizontal cover of tree crowns and 216 circular research plots (plot size 3.14 m², grid intersection 10 × 10 m) established to survey natural regeneration; the longer side of PRP follows the highest counter line; (c) intersection of Pannonian and mountain climate together with large stones and stony debris necessitated rich and diversified tree species composition of beech-oak old-growth forests in NNR Sitno.

Gaps’ characteristics were described in terms of relative frequencies of gaps of different size classes, proportion of gap area on the study area and the number of gapmakers per gap and the degree of their decomposition according to Albrecht [34]. Gaps were further categorized based on the type of their enlargement (dynamics) into three categories: 1—no enlargement; 2—past enlargement; 3—recent enlargement. Gap openings originating from simultaneous death of one or more gapmakers with the identical degree of decomposition were included in enlargement category one. The second category was represented by gaps comprising two and more gapmakers of varying degree of decomposition, but non-including the first degree of decomposition. The third category represented gaps, in which gapmakers had varying degree of their decomposition including also the first degree.

2.3. Data Analysis

To evaluate the changes in the disturbance regime on the monitored PRP, standard descriptive statistics of species composition, density, basal areas and volume of living trees, deadwood, and natural regeneration (seedlings, saplings, and young trees) were calculated in statistical package Statistica 12.0 [35]. The diameter distributions of individual species in mature stand were obtained by sorting of individual diameters and standard size and variability characteristics were calculated (averages, standard deviations). Similarly, the gap size and gap enlargement frequency distributions and simple descriptive characteristics of gapmakers’ features were provided with support of software Statistica 12.0 (StatSoft, Inc., Tulsa, OK, USA).

To analyze the impact of disturbance regime on natural regeneration, the absolute and relative density (abundance) of particular species was evaluated under canopy and in gaps respectively. Differences in absolute numbers (densities) of main species between closed canopy and expanded gaps were tested by Mann–Whitney U test separately for each regeneration category (e.g., seedlings, saplings). The effect of gaps on regeneration was also examined by employing ratio calculated as
absolute density of species individuals in gap relative to density in the control undisturbed area (under canopy). One-way ANOVA was applied to test if significant differences exist in the absolute density of seedlings, saplings and young trees (separately for dominant species) according to gap enlargement categories (none, recent, past).

To determine how much of variation of tree species abundances on sampling plots can be (uniquely) attributed to (i) variation of environmental gradients formed by gaps and density of forest stand surrounding sampling plots, and (ii) spatial variation of environmental conditions on sampling plots across the PRP, we applied two ordination analyses—detrended correspondence analysis (DCA) and redundancy analysis (RDA). Software package CANOCO 4.5 [36] for ordination analyses and the visualization of the results was used.

Tree species densities of natural regeneration on sampling plots (size 1 m$^2$, $n = 216$) entered the analyses as dependent variables separately for each species and two regeneration categories—seedlings (Sdl) and saplings (Sap). Values of tree species absolute densities were transformed by decadal logarithm ($\log_{10}(n + 1)$) to approach normality assumption. The category of young trees was not considered because dataset was dominated by zero values and only two tree species accounted for reasonable numbers allowing multiple comparisons. The plot–species matrix containing 14 dependent variables in total (abundancies of 7 species × 2 regeneration categories on 216 sampling points) was prepared.

Two sets of explanatory variables were used as independent variables characterizing (i) the stand, gap, and gap-size related partitioning of environmental conditions on environmental gradient between the center of gap and closed stand (environmental variables) and (ii) spatial variation of environmental regeneration conditions among sampling plots within the stand (spatial variables). Environmental variables included: (i) three dummy variables of plot presence or absence in open gap, expanded gap, and close canopy conditions, (ii) size of expanded gaps (gap size), and (iii) sum of basal areas (SumBA) and crown projection areas of trees (SumCro) extracted from the 5 m buffer zone surrounding each circular sampling plot. Set of spatial variables was derived from $x$ and $y$ coordinates (by their multiplication or power transformation) of each sampling plot: $x, y, x^2, xy, y^2, x^3, x^2y, xy^2$ and $y^3$ in accordance with the methodology for variation partitioning suggested by Borcard et al. [37]. The plot-environmental matrix consisting of 15 environmental/spatial predictors in total (6 environmental variables + 9 spatial variables on 216 sampling plots) was elaborated.

The variables in plot-environmental matrix entered the RDA as the set of explaining, predictor variables for set of species absolute densities (involved in plot-species matrix) defining abundance and species composition of natural regeneration.

DCA as indirect gradient method had confirmation character and verified the selection of RDA as valid method of linear ordination. Detrended correspondence analysis (DCA) applied on both independent and dependent variable sets revealed the length of the longest ordination axis was 2.789. This fact suggests, the studied environmental gradients are short and as such, it allows to apply linear approximation of species response.

Subsequently, following the methodology of Borcard et al. [37], variance partitioning [37,38] through partial RDA-analyses was used to identify relative proportion of environmental and spatial factors—individually or jointly—on the amount of explained variation of tree species density, separately for seedlings and saplings. For this purpose, forward selection of explanatory variables was employed, only the variables with $p < 0.05$ were selected. Species densities were centred. To test statistical significance of individual variables and models, we used Monte-Carlo test of permutation. As our aim was also to reveal the spatial influences despite the sampling plots were placed in the grid, we used 999 unrestricted permutations [36]. Besides the variation portioning, the RDA allow to reveal the most influential environmental and spatial factors explaining significant amount of variation in dependent set of absolute species densities.
To study the responses of individual species in more detail, we built and tested the general linear models (GLM). GLMs were used to evaluate the relationship between log-transformed density of each tree species in natural regeneration ($\log_{10} (n + 1)$) and set of environmental factors. Main intention was to reveal the differences in sets of environmental variables allowing intentionally supporting the regeneration and survival of some tree species over other ones. Thus, the GLM models can provide useful information applicable for promotion of the species diversity in managed beech-oak stands.

3. Results

3.1. Description of the Stand

Forests with basal area of 39.8 m$^2$ ha$^{-1}$ and stem density of 603 trees ha$^{-1}$ cover the study site and include the presence of nine tree species (Table 1). The canopy layer is dominated by *A. pseudoplatanus*, *F. sylvatica*, and *F. excelsior*. Together with *A. platanoides* the dominant species attain more than 75% of total tree number. Other more represented tree species are *C. betulus* and *T. cordata*, with relative density of 7.8% and 9%. *Q. petraea* has proportion only 3.5%.

| Tree Species       | Density | Basal Area |
|--------------------|---------|------------|
|                    | N ha$^{-1}$ | % | m$^2$ ha$^{-1}$ | % |
| *Acer platanoides* | 17      | 2.8        | 1.8             | 4.6   |
| *Acer pseudoplatanus* | 210   | 34.8       | 7.6             | 19.1  |
| *Fraxinus excelsior* | 99     | 16.4       | 3.2             | 7.9   |
| *Fagus sylvatica* | 135     | 22.4       | 15.3            | 38.4  |
| *Tilia cordata*    | 54      | 9.0        | 3.2             | 8.1   |
| *Ulmus scabra*     | 18      | 2.9        | 0.7             | 1.6   |
| *Carpinus betulus* | 47      | 7.8        | 3.2             | 8.1   |
| *Quercus petraea*  | 20      | 3.4        | 3.6             | 9.2   |
| *Abies alba*       | 3       | 0.5        | 1.2             | 3.0   |
| **Total**          | 603     | 100.0      | 39.8            | 100.0 |

The total deadwood volume represents 115 m$^3$ ha$^{-1}$ (Table A1). Overall, diameter distribution of living trees on the PRP shows continuous recruitment and resembles J-shape form, indicating continuous establishment of trees (Figure 2a). Diameter distributions of *A. pseudoplatanus* and *F. excelsior* exhibit peaks in the lowest diameter classes (Figure 2b), which indicate their successful contemporary establishment. *F. sylvatica* unlike the *A. pseudoplatanus* and *F. excelsior* shows continuous recruitment with the widest range of diameter classes, but with decreased number of trees in the lowest diameter class, what can be regarded as the early sign of difficulties in establishment. Diameter distribution of *Q. petraea* shows peak in middle classes and the absence of recruitment in low diameter classes indicating inability to establish for a longer time.
Figure 2. Diameter distributions of (a) all and (b) individual tree species on the permanent research plot Sitno (trees with dbh >4 cm). AcPs—A. pseudoplatanus, AcPl—A. platanoides, FrEx—F. excelsior, FaSy—F. sylvatica, TiCo—T. cordata, UlSc—U. scabra, CaBe—C. betulus, QuPe—Q. petraea, AbAl—A. alba.

3.2. Description of the Disturbance Regime

Based on the field mapping of the whole area, we identified 25 gaps, representing on average 10 gaps per hectare (Figure 3). Almost 60% of the PRP is covered by gaps (open canopy gaps—22.8%, expanded gaps—59.5%). Small expanded gaps (100–300 m²) represent 28% of the total number of gaps and 9% of the total area of gaps, large expanded gaps (300–1000 m²) account for 64% and 62% of the gap area, and very large expanded gaps (>1000 m²) account for 8% of the number of gaps and 29% of the gap area respectively (Figure A1).

Mean size of open gap is 228 m² and ranges from 14–1694 m² and mean size of expanded gap is 595 m² and ranges from 94 to 3221 m². Natural regeneration covers significantly smaller area than open or expanded gaps (18.1%) because some gaps are filled by the next generation of young trees (Figure A3). The smaller than 250 m² regeneration polygons prevail (Figure 3).
From the total expanded gap area, the largest proportion is represented by the recently enlarged gaps (approximately 50%, Figure 4a). There, within a single gap, the first and some other or all other degrees of decomposition of gapmakers are presented. The median gap size of recently enlarging gaps is higher than the rest of categories, where difference is almost significant ($F (2; 22) = 2.94; p < 0.07$). All of gaps in none enlargement category have been formed in the past. Any gap created solely by gapmakers of 1st degree of decomposition exists. This indicates that all gaps on the PRP have originated in the more distant past, but 50% of them have been expanded recently.

![Figure 3](image-url)

**Figure 3.** Spatial distribution of open and expanded gaps and of polygons of mapped natural regeneration on the permanent research plot Sitno.

Totally, 169 gapmakers are recorded on the sampling plot, 46% and 44% of gapmakers are in the 3rd and the 4th degree of decomposition. Large ratios of gapmakers with high degree of decomposition can be considered as additional evidence that majority of gaps have originated in more distant past.

The most frequent species among gapmakers are *Q. petraea* and *F. sylvatica* (Table 2, Table A1). If we take into account only the numbers of identified gapmakers, proportion of *Q. petraea* in the past species composition is approximately 50% and proportion of *F. sylvatica* is approximately 25%. The number of gapmakers on single gap ranged from 1 to 37, accounting on average for seven gapmakers. Almost
50% of gaps originated from fall of less than five gapmakers, approximately one third of gaps were created either by one or four gapmakers (equally 17%, Figure A2).

| Table 2. Species composition of gapmakers according to degree of decomposition. |
|---------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Tree Species              | Degree of Decomposition |
|                          | 1     %  | 2     %  | 3     %  | 4     %  | Total %  |
| Acer pseudoplatanus       | 0    0.0 | 0    0.0 | 7    9.0 | 0    0.0 | 7     4.1 |
| Fagus sylvatica           | 4   33.3 | 4   80.0 | 10   12.8 | 2    2.7 | 20    11.8 |
| Quercus petraea           | 4   33.3 | 0   0.0 | 31   39.7 | 6    8.1 | 41    24.3 |
| Tilia cordata             | 1    8.3 | 0   0.0 | 0    0.0 | 0    0.0 | 1     0.6 |
| Fraxinus excelsior        | 1    8.3 | 0   0.0 | 1    1.3 | 0    0.0 | 2     1.2 |
| Carpinus betulus          | 2   16.7 | 1   20.0 | 1    1.3 | 1    1.4 | 5     3.0 |
| Abies alba                | 0    0.0 | 0   0.0 | 1    1.3 | 2    2.7 | 3     1.8 |
| Undetermined              | 0    0.0 | 0   0.0 | 27   34.6 | 63   85.1 | 90    53.3 |
| Total                     | 12   100.0 | 5  100.0 | 78  100.0 | 74  100.0 | 169  100.0 |

3.3. Regeneration

Total density of regeneration is 69282 stems ha\(^{-1}\). *A. pseudoplatanus* and *A. platanoides* are the most abundant species in the regeneration layer, with relative density of seedlings between 25%–50% and saplings between 40–50% from the total number of individuals (Table 3). The relative frequency of occurrence on sampling plots for *Acer sp.* is even higher ranging between 35–80%. The *F. excelsior* has relative density 13% in seedlings and saplings, and frequency of occurrence on sampling plots between 35–55%. Proportion sum of all these three species on the tree number of regeneration (near to 95% in the seedling and near to 90% in the sapling category) markedly surpass the proportion sum on tree number of main upper layer, which is 54%.

| Table 3. Density of natural regeneration on the permanent research plot Sitno. |
|-------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Tree Species              | Seedlings (<50 cm) | Saplings (>50 cm) | Young Trees (4–8 cm) |
|                             | Density Freq % | Density Freq % | Density Freq % | Density Freq % |
| N ha\(^{-1}\)     | % | N ha\(^{-1}\)     | % | N ha\(^{-1}\)     | % | N ha\(^{-1}\)     | % |
| Acer platanoides           | 12,901 25.9 | 69.9 | 5116 26.1 | 43.1 | 2.4 | 1.0 |
| Acer pseudoplatanus        | 27,925 56.2 | 77.3 | 9952 50.9 | 49.0 | 149.6 | 59.5 |
| Fraxinus excelsior         | 6885 13.8 | 54.6 | 2654 13.6 | 35.6 | 74.0 | 29.4 |
| Fagus sylvatica            | 1032 2.1 | 17.1 | 265 1.4 | 6.5 | 6.0 | 2.4 |
| Tilia cordata              | 605 1.2 | 6.9 | 693 3.5 | 6.9 | 12.0 | 4.8 |
| Ulmus scabra               | 177 0.4 | 3.7 | 722 3.7 | 9.7 | 5.2 | 2.1 |
| Carpinus betulus           | 192 0.4 | 3.2 | 147 0.8 | 4.2 | 1.2 | 0.5 |
| Quercus petraea            | 0 0.0 | 0 | 15 0.1 | 0.5 | 0 | 0.0 |
| Total                      | 49,717 100.0 | 19,565 100.0 | 251.6 100.0 |

*Freq—frequency of occurrence.*

*F. sylvatica*, *T. cordata*, *U. scabra*, and *C. betulus* are presented in both seedlings and saplings categories only marginally (0.5–3%), although the frequency of their occurrence is somewhat higher (3–17%). Their proportion sums are 4% in the seedling and 9% in the sapling category. That fact is that in contrast with their proportion on the main tree layer 41%—these species are clearly under-represented in regeneration layer.

None of *Q. petraea* seedlings has been recorded on the sampling plots, and density of saplings is sparse. *Q. petraea* in regeneration is absent although its proportion in the tree number of main layer is still 3.5%. Overall, there is a significant disproportion between the expected tree species composition corresponding to the habitat, the current tree species composition of the main tree layer and the tree species composition of the next generation.
3.4. Impact of Gaps on Density and Species Composition of Regeneration

The absolute density of seedlings in gaps is lower than the density under closed canopy on average by 16% (Table 4). Among the most abundant tree species, F. excelsior is the only species, which seedlings significantly profit from growing under canopy (Mann–Whitney U test; p < 0.05). In later regeneration categories, gaps significantly increase the regeneration density of all saplings by 77% and of young trees by 545%. Differences between densities of A. pseudoplatanus and F. excelsior saplings in gaps compared to canopy are statistically significant.

Table 4. Tree species density of natural regeneration in expanded gaps and under closed canopy.

| Tree Species     | Seedlings (≤50 cm) | Saplings (>50 cm) | Young Trees (4–8 cm) |
|------------------|--------------------|-------------------|----------------------|
|                  | N ha\(^{-1}\) Gap | Ratio %           | N ha\(^{-1}\) Gap | Ratio %         | N ha\(^{-1}\) Gap | Ratio % |
| A. platanoides   | 15,014 11,363 76   | 4270 5732 134     | 0.8 1.6 200         |
| A. pseudoplat.   | 28,522 27,490 96   | 6264 * 12,637 * 202 | 6.0 143.6 1396 |
| F. excelsior     | 8924 * 5401 * 61  | 1540 * 3465 * 225 | 20.4 53.6 340 |
| F. sylvatica     | 1225 892 73       | 105 382 364       | 4.0 2.0 50         |
| T. cordata       | 840 433 52        | 875 561 64        | 2.0 10.0 500       |
| U. scabra        | 210 153 73        | 455 917 202       | 0.8 4.4 550        |
| C. betulus       | 105 255 243       | 35 229 654        | 0 1.2 -            |
| Q. petraea       | 0 0 -             | 0 25 -            | 0 0.0 -            |
| Total            | 54,840 45,987 84  | 13,544 23,949 177 | 34 218 645        |

Tree species with relative density or frequency of occurrence above 10% are marked bold; *—Mann–Whitney U test; \(p < 0.05\).

Under the canopy, relative densities of tree species more differ among regeneration categories than in gaps (Figure 5a). A. pseudoplatanus and platanoides show big decrease in relative density of young trees under the canopy. Other less presented species are even absent in the category of young trees under canopy, i.e., they are not able to survive. Oppositely, young trees of F. excelsior and F. sylvatica show increased densities indicating their better contemporary ability to survive the initial regeneration stages compared to all other species. Two species can grow and survive under canopy for a longer time—F. excelsior and F. sylvatica—under contemporary natural conditions.

Figure 5. Relative density of seedlings, saplings and young trees under (a) closed canopy and (b) in expanded gaps.
In expanded gaps, relative densities of tree species remain stable across all regeneration categories, except *A. platanoides* (Figure 5b). The ingrowth into young trees show *A. pseudoplatanus* and *F. excelsior*. Relative densities of other species remain stable in different regeneration categories, i.e., other species sustain their presence reached as seedlings.

In summary, *F. excelsior* and *F. sylvatica* manifest higher absolute and relative regeneration densities and good ability to survive severe competition in initial developmental stages. *A. pseudoplatanus* regenerates in very high density, but successfully survives only in gaps. Similarly, *A. platanoides* regenerates in very high densities, but irrespective whether it grows in gap or under the canopy. The lower competitive ability on the given site severely limits its ingrowth into upper tree layers. Other species show very limited ability to regenerate (low absolute and relative densities) and they grow into the category of young trees only in gaps.

### 3.5. Impact of Environmental and Spatial Factors on Natural Regeneration

Relationships between environmental factors, spatial variables and tree species densities revealed by linear RDA ordination are provided on Figure 6. The position of environmental variables in ordination space allows to interpret the ordination axis as gradients: (i) horizontal axis X is strongly positively correlated with variable canopy and at the same time strongly negatively with dummy variable open gap that suggest interpretation as light-moisture gradient between the closed canopy and open gap. The increase of *x* ordination coordinates means decrease of light and increase of moisture on environmental gradient formed on open gap—closed canopy link; (ii) vertical axis Y is positively correlated with *x* and *y* coordinates of sampling plots. Movement across the PRP in the direction of increasing spatial coordinates *x* and *y* means movement along the longer side of the transect (spatial gradient). A significant correlations of species densities with second ordination axis means that variation of environmental conditions along longer side of PRP significantly affect the variation of species densities—density can increase or decrease along investigated direction.

On the Figure 6, the arrows of variables depict the magnitude of correlations of individual variables with ordination axis. The close position of different variables in the same sector of ordination space means not only similar relationships with ordination gradients, but also strong mutual correlation.

The seedlings’ densities of the most abundant species *Acer* sp., *F. excelsior*, and *F. sylvatica* and sum of crown projections show positive correlation with environmental gradient—the increase of canopy closure and lower distance from closed canopy (source of seeds) support their density in the initial regeneration category. The gap size and sapling density of *A. pseudoplatanus* manifests the negative correlations with environmental gradient and due to close position, they are positively correlated. The decreasing canopy closure and increasing gap size support the survival of *A. pseudoplatanus* saplings. A similar, although weaker trend is characteristic also for saplings of *U. scabra*, *C. betulus*, and *A. platanoides*.

In the same time, the densities of saplings and seedlings of *F. sylvatica* and *Acer* sp. show stronger negative correlation with spatial gradient. That means the densities are decreasing with the increasing distance along longer side of the transect and they are affected by some systematic spatial trends in environmental conditions or stand characteristics on the PRP. *F. excelsior* shows almost no spatial trends, suggesting its ability to regenerate and survive in a wide range of natural conditions. Other less abundant species and saplings of *F. excelsior* depend on environmental and spatial gradients only very marginally. Slight supportive effects of decreasing shading and short distance position on the transect of sapling density of *Ulmus*, *Carpinus*, and *A. platanoides* is observable.
The common variability explained by both spatial and environmental variables of around 2% (Figure 7) could be attributed to spatial trends of one or more stand or gap characteristics (basal area, crown projections, or number and size of gaps, etc.).
Table 5. Amount of variation of tree species compositions explained by environmental and spatial factors in different RDA variants in the old-growth forest Sitno.

| Factors          | Covariables | F      | p    | %    |
|------------------|-------------|--------|------|------|
| **Seedlings**    |             |        |      |      |
| Envir + Spatial  | None        | 5.252  | 0.001| 18.7 |
| Envir            | None        | 2.549  | 0.018| 3.5  |
| Spatial          | None        | 7.373  | 0.001| 17.5 |
| Envir            | Spatial     | 1.009  | 0.439| 1.2  |
| Spatial          | Envir       | 6.409  | 0.001| 15.2 |
| **Saplings**     |             |        |      |      |
| Envir + Spatial  | None        | 4.814  | 0.001| 17.9 |
| Envir            | None        | 4.754  | 0.001| 8.3  |
| Spatial          | None        | 5.425  | 0.001| 11.4 |
| Envir            | Spatial     | 4.062  | 0.001| 6.5  |
| Spatial          | Envir       | 4.841  | 0.001| 9.6  |

Models comprise all variables (factors) selected by forward selection in RDA separately for Envir. and Spatial group of variables (see Table 3); F—the value of the test criterion; p < 0.05 is marked bold; %—relative proportion of variation explained by the model.

Table 6. The relative amount of variation of tree species composition explained by selected environmental and spatial factors (forward selection) in the RDA analysis in the old-growth forest Sitno.

| Group of Factors | Seedlings Variable/Factor | % | p     | Saplings Variable/Factor | % | p     |
|------------------|---------------------------|---|-------|--------------------------|---|-------|
| **Envir.**       | gap size                 | 2 | 0.020 | SumCro                   | 4 | 0.001 |
|                  | Canopy *                 | 1 **| 0.018 | canopy                   | 2 | 0.017 |
|                  |                           |   |       | SumBA                    | 2 | 0.006 |
| **Total**        |                           | 3.5| 0.018 |                         | 8.3| 0.001 |
| **Spatial**      | X                         | 4 | 0.003 | Y^3                      | 3 | 0.002 |
|                  | X^2                      | 6 | 0.001 | Y                        | 4 | 0.001 |
|                  | X^3                      | 2 | 0.002 | X^2Y                     | 1 | 0.006 |
|                  | XY^2                     | 2 | 0.006 | Y^2                      | 2 | 0.008 |
|                  | Y^3                      | 2 | 0.018 | X^3                      | 1 | 0.040 |
|                  | Y                        | 1 | 0.010 |                          |   |       |
| **Total**        |                           | 17.5| 0.001|                         | 11.4| 0.001 |

(*) as variable canopy is one of three categories coding the type of canopy closure, variables expgap and opengap entered the analysis automatically without further testing. (**) % of explained variability for selected variables (factors) representing conditional effect, i.e., it is the percentage of explained variability for individual variables, when simultaneously used the earlier selected variables as covariables. p < 0.05 is marked bold.

Figure 7. Variation partitioning between environmental (Env.) and spatial factors (Spt.). Venn diagrams display how much tree species variation of (a) seedlings and (b) saplings can be uniquely attributed to environmental (gap related) factors and how much variation they have in common.
To assess the influence of environmental variables on natural regeneration in more detail, multiple linear regressions of log-transformed densities are tested separately for each tree species (Table 7).

Table 7. Regression β-coefficients obtained from general linear models for each individual tree species and height growth category of natural regeneration in relation to independent environmental factors, displayed are R-squares of the whole models and their p-values.

| Tree Species | Gap Size | SumCro | SumBA | Plot Position | R² (%) | p   |
|--------------|----------|--------|-------|---------------|--------|-----|
| A. pseudoplatanus | 0.12    | 0.16   | −0.09 | 0.14 | 3.08 | 0.254 |
| A. platanoides   | 0.17    | 0.17   | −0.15 | 0.23 | 5.22 | 0.046 |
| F. excelsior     | −0.23   | 0.03   | −0.03 | 0.04 | 6.74 | 0.012 |
| F. sylvatica     | 0.14    | 0.12   | −0.16 | 0.15 | 3.13 | 0.245 |
| T. cordata       | 0.00    | 0.16   | −0.08 | -   | 1.90 | 0.545 |
| U. scabra        | 0.11    | −0.10  | 0.08  | 0.14 | 2.01 | 0.509 |
| C. betulus       | −0.03   | −0.10  | −0.09 | -   | 4.73 | 0.069 |

Seedlings

| Tree Species | Gap Size | SumCro | SumBA | Plot Position | R² (%) | p   |
|--------------|----------|--------|-------|---------------|--------|-----|
| A. pseudoplatanus | 0.08    | −0.43  | 0.31  | −0.18 | 14.08 | 0.000 |
| A. platanoides | 0.05    | −0.22  | 0.07  | 0.04  | 3.14  | 0.243 |
| F. excelsior   | −0.21   | −0.24  | 0.15  | −0.29 | 9.83  | 0.001 |
| F. sylvatica   | 0.25    | −0.06  | 0.02  | 0.07  | 4.75  | 0.069 |
| T. cordata     | −0.11   | 0.20   | −0.08 | −0.14 | 3.76  | 0.153 |
| U. scabra      | 0.07    | −0.14  | −0.10 | −0.01 | 6.37  | 0.017 |
| C. betulus     | −0.14   | −0.12  | −0.01 | −0.19 | 4.63  | 0.076 |

R² (%) = R-square × 100; p < 0.05 is marked bold.

Results confirm and underline several facts revealed by RDA ordination and by variance partitioning:

(i) environmental factors have greater impact on the density of saplings compared to seedlings—documented by higher percentage of explained variability and higher statistical significances of individual GLM models in the sapling category,

(ii) in the category of seedlings, the gap size and under canopy position (as revealed in the RDA) have a predominant effect, which is also approached by the sum of crown projections for the prevailing tree species Acer sp., F. excelsior, F. sylvatica—documented by higher absolute values of standardized beta coefficients,

(iii) in the category of saplings, the amount of crown projections and under canopy position—again documented by the higher absolute values of the standardized beta coefficients—have a predominant effect (in accordance with the RDA).

However, unlike the RDA, GLM models also allow evaluation of the nature of linear impact and allow the development of individual tree profiles.

From this perspective, it is important in seedlings’ categories that:

(i) signs of beta coefficients of canopy position, gap size and sum of crown projections are predominantly positive—under canopy position, increasing gap size and sum of crown projections have positive influence on species densities;

(ii) The previous statement fully applies to Acer sp. and F. sylvatica, but not for F. excelsior. Here, the presence or absence of under canopy position and sum of crown projections loses its influence and, in addition, the gap size has a negative effect—the larger the gap area, the lower the density of the natural regeneration of F. excelsior.
In saplings’ category GLM indicate that:

(i) under canopy position and increasing sum of crown projections reduce the density of regeneration (F. excelsior is also negatively affected by the increasing size of gaps)

(ii) the previous statement fully applies to A. pseudoplatanus and F. excelsior and partly also to A. platanoides, but not to F. sylvatica. The only significant environmental variable for Fagus, is the gap size. As a result, the larger the gap area, the better survival of Fagus seedlings.

4. Discussion

4.1. Natural Regeneration

Small-scale disturbances maintain the old-growth forest structure mainly through the emergence and survival of natural regeneration in the variety of conditions in canopy openings. Total density as well as tree species composition of natural regeneration in species-rich old-growth forest in NNR Sitno corresponds well with the abundance found in other broadleaved-dominated, mixed old-growth forests of the Western Carpathians (e.g., [39]). In tree species composition, Acer species (A. pseudoplatanus and A. platanoides) are the most abundant except the category of young trees under the canopy. Acer sp. achieve a high density of regeneration in the positions under the canopy near larger gaps and for survival they need decreased shading achieved mainly through the lower sum of crown projections. A. pseudoplatanus, however, unlike A. platanoides clearly survives well in open gaps. F. excelsior has the highest relative share in the category of young trees under the canopy and the second highest in the same category in gaps. F. excelsior regenerates and survives well especially in smaller gaps, although in later ages it demands lower sum of crown projections and prefer expanded or even open gap position.

Considering the density of (potentially) seed-producing F. excelsior stems, i.e., the canopy trees with dbh over 20 cm comprising about 10%, the abundance of F. excelsior seedlings and saplings is over-proportionately high. F. sylvatica regenerates especially under the cover of the mature stand near larger gaps, but in a later developmental stage of saplings it may survive better in larger gaps. T. cordata, U. scabra, and C. betulus reveal less clear pattern. These species show the very limited ability to regenerate (low absolute and relative densities) and they ingrow into upper stand layers only in gaps. The higher number of tree species is able to survive initial regeneration stages and ingrowth into the category of young trees in gaps.

Natural regeneration of Q. petraea is scarce in the long-term. Saniga et al. [40] have reported the lack of Q. petraea individuals above the height of 80 cm on all permanent research plots established in NNR Sitno, with the maximum density of natural regeneration 667 individuals per hectare since the late 80-ties of the previous century. Species composition as well as abundance and height structure of natural regeneration are affected not only by gap properties, but also by other factors that are relevant for the survival and height growth of seedlings and saplings. The influence of herbivory may limit the competitive strength of particular species and possibly to change tree species composition [41–43]. Therefore, we assume that especially regeneration of light-demanding oak suffers under repeated browsing and is unable to grow out of its competitors.

4.2. Disturbance Regime

Many studies are devoted to gap dynamics and to the undoubted effect, which canopy gaps have on regeneration density [44]. Forest management aimed at the importance of diverse forests relies on that natural disturbances are key drivers of promoting and maintaining tree species diversity [45]. Gap dynamics of mixed beech-oak old-growth forests characterized by diverse composition of canopy layer are less frequently studied in Central Europe [11,31,46,47] and provide fragmented base of scientific information. Canopy layer of investigated preserved forest remnant consists of nine tree species with contrasting light requirements (F. sylvatica, A. pseudoplatanus, Q. petraea, F. excelsior, T. cordata, A. platanoides, A. alba, C. betulus, and U. scabra) and possess relatively higher basal area (39.8 m² ha⁻¹) and stem density of trees (603 trees ha⁻¹). In the investigated forest, mid-scale disturbances (16 expanded
gaps >300 m$^2$) prevail with a low occurrence of disturbances corresponding with very large gaps (two expanded gaps >1000 m$^2$). Disturbance regime and to this related light availability may thus favour tree species of less shade (as *A. pseudoplatanus* or *F. excelsior*) and intermediate shade tolerant species (*Q. petraea*) to regenerate, establish, grow, and recruit into the forest canopy [7,23,48].

Large proportion of stand area (up to 60%) is under gaps created by the mortality of canopy trees. The mortality of both *Q. petraea* and *F. sylvatica* drives the disturbance regime of NNR Sitno, which are the main gapmakers and represent the highest density and basal area among dead trees.

Our results clearly indicate the significant shift in species composition over the time. The Quercus has declined in the recent period. Additionally, massive spread of *F. excelsior* and associated decrease of *F. sylvatica* dominance is registered. Results point to significant disproportion between the expected tree species composition corresponding to the habitat, the current tree species composition of the main tree layer and the tree species composition of the next generation.

In the long-term (over 30 years of inventory) the relative density of adult living trees in tree species composition decreases for both *Q. petraea* and *F. sylvatica*, but more rapidly for *Q. petraea* [40]. This agrees with the study showing increased mortality during 50 years of natural forest development for both *Q. petraea* and *F. sylvatica*, but stronger for *Q. petraea* in Swiss lowlands [30]. The study from Romanian oak-beech forests also support our results declaring disturbance regime is primarily driven by the mortality of *Q. petraea*, showing the absence of this species in the process of tree recruitment [31].

The largest number of gapmakers is represented by lying deadwood in advanced stage of decomposition (89%), while recently dead trees represent only 7%. Wind has an important role in creating gaps by single or by consecutive disturbance events (only 17% trees died standing). The group mortality prevails in the process of gap formation.

Progressive mortality of trees in separate canopy gaps is the typical feature. Successive extensions of more than a half of gaps resulted in formation of large and very large gaps, which is in agreement with reports from mixed beech forests [49]. Higher proportion of progressively enlarged gaps (above $2/3$) is reported in mixed old-growth oak forests of NNR Kašivárová [47]. Differences in gap dynamics of beech-oak forest in NNR Sitno (lower frequency of small gaps, absence of very large gaps, and lower proportion of stand area under gaps) in comparison to mixed oak old-growth forest Kašivárová could be attributed to the fact that trees in NNR Sitno grow on less productive volcanic site with high share of rocks. Interestingly, distribution of expanded gaps in NNR Sitno does not follow negative exponential form described in other temperate oak and beech forests [13,15,31,47], probably due to the recent lack of small gap-scale disturbances. No single gap without extension has been formed recently, suggesting recent changes in disturbance regime.

### 4.3. Gap Partitioning

The distribution of natural regeneration corresponds with the distribution of gaps, which agrees with gap-recruitment studies [44]. Our results suggest that wind disturbances creating successively expanding large gaps do allow maintaining of mid- and late-successional tree species and do not allow early-successional ones and ground vegetation to hamper the regeneration process. Originally, gap partitioning hypothesis assumes that the variety of micro environmental conditions along the gradient shaded understory—canopy gap enable co-existence of several tree species partly because of higher stem density in gaps [48]. Different tree species supposedly have different probability of establishing seedlings in particular range of gap sizes [48].

However, as shown in several studies, gaps not necessarily provide primary environment for regeneration e.g., [32,50]. Our results, the higher regeneration density of seedlings observed under the forest canopy compared to gaps, the continuous recruitment, and narrow and linear ordination gradient confirmed by DCA, which suggests relatively homogenous tree species composition on the most plots, confirm these later studies.

The influence of environmental gradient between closed canopy and gap (denoted also as gap-partitioning) in seedling category is not evident for any of the investigated tree species.
The variability that can be uniquely attributed to gap and stand related environmental factors is very low. The species composition under the canopy and gaps is very similar—all species can establish also under canopy, even in higher densities due to the proximity of seed trees. At seedling stage (≤50 cm), species relationships and abiotic soil related factors have probably the higher importance than distribution of environmental conditions along stand-gap gradient [51].

On the other hand, the relative importance of tree species interactions changes with regeneration developmental category [51]. Our results suggest that environmental gradient is manifested through differential survival rate of different species in saplings and young trees phase. The position under canopy and increasing sum of crown projections reduce the density of saplings. The environment within the gap has significant impact on the tree species composition and positively influences four out of eight studied tree species in sapling category (>50 cm), in agreement with original gap-partitioning theory [48].

Under the assumption that fluctuations in light/moisture availability and spatial variation in disturbance allow limited number of tree species to coexists [23], the two most successful gap fillers A. pseudoplatanus and F. excelsior and recent shifts in disturbance regime suggest future changes in tree species composition in the investigated nature reserve.

The probability of establishment of intermediate shade tolerant tree species increases with increasing of gap size [37]. The presence of two very large gaps (above 1000 m²) does not create sufficient conditions for Q. petraea regeneration. Complete lack of regeneration along with the high mortality of old-grown Q. petraea trees underlines its decline in NNR Sitno. Quercus can successfully establish in gaps of sizes between 300–500 m², however for further successful growth, saplings require gaps of approximately 1000 m² [11] or even more than 2000 m² [52].

To emphasize the contrasting light requirements of oak in comparison to more shade tolerant species, dendroecological study from oak-beech old-growth forest in Romania demonstrates that the oak successfully establishes after stand replacing disturbance [29]. This is analogous with establishment of other intermediate shade-tolerant species as M. obovata in mixed beech forest in western Japan showing patterns suggesting the ability to establish following the large-scale catastrophic disturbance [53]. In the process of recruitment into the canopy, oak unlike beech is less able to respond with multiple releases to repeating canopy openings. In addition, regeneration of oak is not able to recover once the light conditions in canopy improve.

Therefore, match in the masting of parent oak trees and canopy disturbance events is needed or release of oak saplings at their very young life stage [4]. It is worth of mentioning that another intermediate shade-tolerant tree species, T. cordata, responds to disturbance regime in greater densities and shows some potential for continuous recruitment. The recent expansion of T. cordata, C. betulus, and A. pseudoplatanus has been observed in the Bialowieża Primeval Forest in Poland [19].

Among less shade tolerant tree species, F. excelsior greatly benefits from the presence in small gaps. Due to its sensitivity to late spring frost, it avoids exposed sites in the centre of large gaps and regenerates in greater densities in small gaps and under the closed canopy possibly in surroundings of gap edges [54]. To gap extension, it responds with increased growth succeeding in gap capturing over the F. sylvatica [55,56].

The highest frequency of large expanded gaps (300–1000 m²) allowed recruitment of less shade tolerant F. excelsior, A. pseudoplatanus, and A. platanoides, which agrees with findings demonstrating that intermediate severity wind disturbances create conditions beneficial for recruitment of less shade tolerant species [57]. The high densities of natural regeneration of F. excelsior and A. pseudoplatanus and patterns of diameter distributions indicating their contemporary establishment suggest that under recent disturbance regime they are the most successful gap fillers. Spatially clumped exclusively in the gap centres, young trees of A. pseudoplatanus display clear pattern of gap-size partitioning i.e., dependency on environmental gradient. Seedlings regenerate in higher densities in large expanded gaps and oppositely, saplings and young trees are completely absent in small gaps and under closed forest canopy.
Similar to our findings, *A. pseudoplatanus* shows evident gap-size partitioning in beech-fir forest reserve in the Dinaric Mountains [58]. Along with the *A. pseudoplatanus*, *A. platanoides* is the second most abundant regenerating tree species. Due to its weak competitive ability [59] it recruits in very low density and frequency of young trees, even lower than for example *U. scabra*. Spatio-temporal changes in disturbance regime could have distinct consequences for gap replacement patterns.

Decreases in growth performance of drought-sensitive *F. sylvatica* [60,61] and recent lack of small gaps may hamper regeneration of shade tolerant tree species *F. sylvatica* on low water retention site. The importance of small gaps illustrates the fact that *F. sylvatica* is the only species, whose young trees responded to gaps by 50% decrease relative to forest canopy. This agrees with low (below 10%) relative density and frequency of *F. sylvatica* seedlings and saplings and with indicated contemporary difficulties in establishment based on the diameter distribution of trees. Young trees of *F. sylvatica* establish under close canopy, prior to gap formation. Based on our results, shade tolerant *F. sylvatica* reveals no clear gap partitioning similar to reports for old-growth beech-oak forests in Northern Spain [62] or beech-fir forests in the Dinaric Mountains [58]. Analogous patterns of *F. sylvatica* establishment—understorey regeneration with successive releases of trees—reports study from the oak-beech forest in Romania [29].

Our findings confirm that in mixed forests with oak, the more shade tolerant tree species than oak have competitive advantage in recruitment [31,63,64]. Under the canopy and to this related low light availability, *F. sylvatica* shows the highest survival, *A. pseudoplatanus* the lowest and *F. excelsior* in between these two species [65]. In expanded gaps with higher light availability, *A. pseudoplatanus* and *F. excelsior* show higher height growth rates and allocate more biomass to their stems than *F. sylvatica*, which allocates more biomass to branches [66]. Different growth strategy points to the ability of *F. sylvatica* adults to expand and close small canopy gaps by lateral growth of branches [67], but saplings and young trees to lose with *Acer* sp., which growth rates can be twofold higher than those of *Fagus* even in small gaps [22,48].

Quantification of relationship between the various gap sizes and the related environment along with the spatial arrangement and the species composition of natural regeneration seems not to be simple. Low amount of extracted variability in our study suggests that a regeneration process is influenced by other factors. Beyond the gap size, many factors and gap-related processes generate the mosaic spatial structure of the regeneration and recruitment in mixed forest stands. Differentiation in the regeneration niche (production of seeds—mast seeding, seed dispersal, germination, and establishment of seedlings [68]), presence, location and abundance of parent trees and seed dispersers, degree of exposed mineral soil and amount of wood debris [69], micro-environmental factors, including position within gaps [48], advance regeneration [70], different life strategies of tree species in accessing the canopy [22], degree of browsing pressure [71] above- and below-ground competition among tree species, and climate change [72], consequently creating new disturbance regimes [73] can be mentioned.

The composition of natural regeneration, its life stage and spatial structure and distribution of gaps might reveal more clear relationships and allow for quantification of higher amount of variability if initial gap size and time since gap formation is related to density of young trees capturing gap. This highlights the future research to concentrate on repeated gap survey studies [67] or to combine the retrospective dendroecological approach and static survey. The studies of disturbance regime in various spatiotemporal scales [6,74] can allow to more accurately predict recruitment of tree species for forest management purposes.

4.4. Implication to Forest Practice

In close-to-nature forest management, the emphasis on stability and continuity of forest ecosystem results in an effort to retain their structural diversity at small spatial scales. The reliance on natural processes, especially the natural regeneration leads to the use of gap-based regeneration as a fundamental silvicultural technique [75]. Small-area shelterwood system successfully uses harvest-created gaps to initiate natural regeneration of shade-tolerant species in mixed forest stands. The negative side of this approach is that size-asymmetric competition eliminates light-demanding or
slow-growing species during the regeneration phase entirely [76]. According to tree species composition and gap size distribution found in our study, regeneration in gaps is dominated by shade-tolerant or intermediate species while light-demanding Q. petraea is rare.

The most common silvicultural practice, to establish and maintain the light-demanding oak recruitment is the regular shelterwood system with short duration of regeneration period. However, recent efforts for minimization of the canopy layer disruption lead to employment of regeneration techniques based on small gaps and complicate thus the oak regeneration considerably [64]. Moreover, advanced regeneration of shade-tolerant Acer sp. and F. excelsior reduces the potential of larger gaps for establishment of Q. petraea regeneration. Therefore, regeneration requirements of all tree species are often not met with harvest-created gaps alone [77].

If light-demanding species should be regenerated, while the mixed ecosystem should be established and maintained, lags in regeneration of shade tolerant species following creation of large gaps (at least 0.2 ha) would create better situation for light-demanding Q. petraea. The selection of shade-tolerant A. pseudoplatanus and platanoides in the first (seed) cutting contribute to reducing their seed bank in favour of oak. After oak is successfully established, its overtopping should be prevented by combination of fast progressing of gaps enlargement and subsequent control of fast-growing Acer sp., Fagus and Fraxinus recruitment.

The results of our study confirm that to regenerate the mixtures of Acer, Fagus and Fraxinus does not represent a complicated task. Subsequent changes in the proportions of separate tree species in the thicket stage (i.e., saplings and young trees in our study) are dependent on the decision of the forest manager, with the possible modifications caused by the other factors, especially by the selective damage. On the other hand, the maintenance of Quercus must be thoroughly planned in advance—already at the identification of suitable conditions for location of the gaps, the determination of their size and the realization of seed cutting therein. The duration of regeneration period should by last 40 years, whereby the duration of regeneration periods within groups should be differentiated: 15 years for Q. petraea and 25 years period for Acer, Fagus and Fraxinus.

5. Conclusions

The multiple-species old-growth forest in NNR Sitno shows stable and sustainable regeneration and recruitment of native tree species. Currently, small-scale wind disturbances, which create progressively enlarged gaps of mainly 300–1000 m², promote regeneration of four out of nine studied tree species. The current disturbance regime negatively affects regeneration of formerly dominant F. sylvatica and seems less suitable for light demanding species as well. The significant shift in species composition over the time is clearly visible. Q. petraea has declined in the recent period, also massive spread of F. excelsior and associated decrease of F. sylvatica is registered. There is a significant disproportion between the expected tree species composition corresponding to the habitat, the current tree species composition of the main tree layer and the tree species composition of the next generation.

Gap size, type of its extension and spatial distribution and gap related stand characteristics have relatively small, but significant impact on natural regeneration. Total explained amount of variation in species composition of natural regeneration (defined as sets of absolute densities on sampling plots) is 18.7% for seedlings ($p < 0.001$) and 17.9% for saplings ($p < 0.001$). Spatial factors have greater influence than gap related factors. The influence of environmental gradient between closed canopy and gap (gap-partitioning) in seedling category is not evident for any of the investigated tree species, but it is manifested in saplings and young trees phase through differential survival rate of different species. The current size and spatial distribution of gaps promote two light less shade tolerant tree species—A. pseudoplatanus and F. excelsior—that recruit into the canopy with the highest relative densities.

The observed development of beech-oak association provides outstanding information about natural processes ongoing on species rich habitats under current environmental change. The gained knowledge can serve as a baseline for design of silvicultural systems aimed to preserve diverse
forests by mimicking natural disturbance dynamics in managed mixed stands growing on similar sites. In this regard, if intermediate light-demanding species should be regenerated to preserve species richness and diversity, the use of more variable (mosaic) shelterwood systems in comparison to contemporary practice must be employed. In seed years of light demanding species, some parts of stand canopy must be disturbed by larger gaps (over 0.2 ha) to form wider environmental gradient and to achieve lag in regeneration of shade tolerant species. The removal of less shade-tolerant (e.g., *Acer* sp., *F. excelsior*) in the first (seed) cutting contribute to reducing their seed bank in favour of intermediate light-demanding less abundant species (e.g., *Q. petraea*). Rapid enlargement of older small gaps with the presence of light more demanding species and/or *F. sylvatica* would be beneficial, whereas the reduction of favourable conditions for *F. excelsior* is likely. Supplementary investment inputs, such as competition control from early growth stages, protection against browsing, game control, etc. are required.

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**Conflicts of Interest:** The authors declare no competing interests.

### Appendix A

**Table A1.** Species composition and basic characteristics of deadwood on the permanent research plot.

| Table         | Density | Basal Area | Volume |
|---------------|---------|------------|--------|
|               | N ha⁻¹  | %          | m² ha⁻¹ | %      | m³ ha⁻¹ | %      |
| *Acer platanoides* | 0       | 0.0        | 0.00    | 0.0    | 0.0     | 0.0    |
| *Acer pseudoplatanus* | 5       | 4.4        | 0.30    | 3.9    | 5.2     | 4.5    |
| *Fraxinus excelsior* | 2       | 1.7        | 0.07    | 1.0    | 1.2     | 1.0    |
| *Fagus sylvatica*   | 16      | 13.9       | 1.32    | 17.4   | 20.0    | 17.4   |
| *Tilia cordata*      | 8       | 7.1        | 0.42    | 5.5    | 5.8     | 5.1    |
| *Ulmus scabra*       | 1       | 1.0        | 0.04    | 0.5    | 0.0     | 0.0    |
| *Carpinus betulus*   | 4       | 3.1        | 0.08    | 1.1    | 1.0     | 0.9    |
| *Quercus petraea*    | 21      | 17.7       | 1.59    | 20.9   | 24.1    | 21.0   |
| *Abies alba*         | 2       | 1.7        | 0.23    | 3.1    | 1.5     | 1.3    |
| Undetermined         | 58      | 49.3       | 3.55    | 46.7   | 56.0    | 48.8   |
| Total                | 118     | 100.0      | 7.60    | 100.0  | 114.8   | 100.0  |

**Figure A1.** Frequency of canopy gaps (light bars) and expanded gap (dark bars) according to gap size classes (calculated as number of gaps in gap size category per total number of gaps).
Young trees (dbh 4–8 cm, about to recruit into the middle stand height layer) of A. pseudoplatanus and F. excelsior dominate canopy openings. A. pseudoplatanus is almost absent in the closed canopy, while young trees of F. sylvatica grow mainly under closed canopy.

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