



# Edge effects on the realised soil seed bank along microclimatic gradients in temperate European forests



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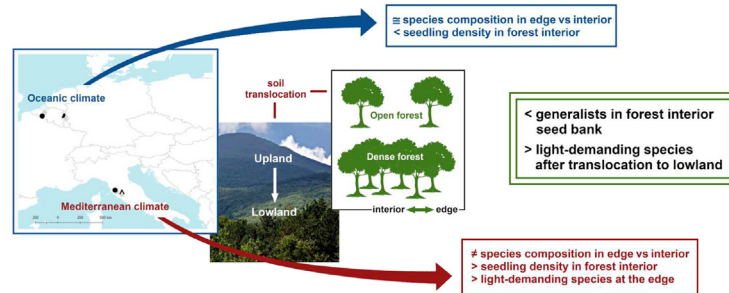
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## HIGHLIGHTS

- Seed bank responses to different microclimates were studied in temperate forests.
- Thermophilous forests showed a compositionally different edge vs interior seed bank.
- Forest edges shifted the seedling community to a more light-demanding composition.
- Soil translocation from up to lowland favoured the emergence of generalist plants.
- Forest fragmentation should be prevented to conserve seed banks' forest specialists.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Despite the crucial role of the seed bank in forest conservation and dynamics, the effects of forest edge microclimate and climate warming on germination responses from the forest seed bank are still almost unknown. Here, we investigated edge effects on the realised seed bank and seedling community in two types of European temperate deciduous forest, one in the Oceanic and one in the Mediterranean climatic region. Responses in terms of seedling density, diversity, species composition and functional type of the seed bank at the forest edge and interior were examined along latitudinal, elevational and stand structural gradients by means of soil translocation experiments. Moreover, we translocated soil samples from high to low elevation forests in the two regions, thus performing a warming simulation. Density, species diversity and mortality of the seedlings varied with region and elevation. Seedling density also differed between forest edge and interior position, while seedling cover mainly depended on forest structure. Both the edge and interior forest seed bank contained a high proportion of generalist species. In Belgium, a more homogeneous seed bank was found at the forest edge and interior, while in Italy compositional and ecological differences were larger: at the forest edge, more light and less moisture demanding seedling communities developed, with a higher proportion of generalists compared to the interior. In both regions, the upland-to-lowland translocation experiment revealed effects of warming on forest seed banks with thermophilization of the realised communities. Moreover, edge conditions shifted the seedling

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composition towards more light-demanding communities. The establishment of more light and warm-adapted species from the seed bank could in the long term alter the aboveground vegetation composition, with communities becoming progressively richer in light-demanding generalists and poorer in forest specialists.

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## 1. Introduction

Species responses often lag behind climate warming and are influenced by local microclimates rather than macroclimate (De Frenne et al., 2019; Zellweger et al., 2020). The buffering of extreme macroclimatic conditions under tree canopies is a worldwide phenomenon (De Frenne et al., 2019), implying that forests are likely to form temporary local microclimatic refugia under globally rising temperatures and during heat waves (Meeussen et al., in press). However, this buffering capacity is severely threatened by increasing forest fragmentation (De Frenne et al., 2021) that creates more forest edges, whose buffering capacity is lower compared to the forest interior (Meeussen et al., in press). Worldwide, at least 20% of the forest area is located within 100 m of a forest edge (ca. 25% for Belgium and Italy) (Estreguil et al., 2013; Haddad et al., 2015). Therefore, a substantial extent of these forests is subject to strong edge influences (Meeussen et al., in press).

The ecological alteration caused by the formation of sudden, natural or artificial edges of forest fragments is defined as edge effects (Landenberger and McGraw, 2004). This effect can likely amplify the negative consequences of climate change (Hofmeister et al., 2019). In fact, local differences in microclimatic conditions (i.e. incoming radiation, wind speed and direction, temperature and humidity) are especially pronounced near forest edges (Chen et al., 1995; Davies-Colley et al., 2000; Meeussen et al., in press). For instance, during the summer, temperature and light levels decrease whereas the relative humidity increases from the forest edge towards the interior (Davies-Colley et al., 2000; Matlack, 1993; Schmidt et al., 2019). This makes forest edges more susceptible to temperature variability, temperature extremes and drought stress compared to interiors (Meeussen et al., in press). Evidence from previous studies shows that magnitude and depth of the edge effect vary with forest structure, as a more extended negative effect in open forests (i.e. for the species richness the distance of the edge influence (DEI) is 35.5 m in open forests and  $\approx 0$  in dense forests) (Aussenac, 2000; Govaert et al., 2020; Meeussen et al., in press). Magnitude and depth of the edge effect vary also with macroclimate, with a stronger impact in warmer climates (i.e. higher depth of edge influence in forests in warm areas) (De Frenne et al., 2019; Lembrechts and Lenoir, 2020; Meeussen et al., in press).

The study of the soil seed bank and its dynamics is critical for understanding forest regeneration and conservation (Dalling et al., 1998; Lin et al., 2006). Indeed, variation in seed bank dynamics is often reflected in the composition, distribution and dominance of above-ground species (Parker and Kelly, 1989; Plue et al., 2017a). The seed bank is the sum of all viable seeds buried in the soil and functions as a memory of current and past plant communities (Simpson et al., 1989). Many biotic and abiotic factors influence the seed bank and elevation, forest composition and structure are considered important drivers of seed bank variations (Luo et al., 2017; Zou et al., 2021). Greenhouse studies quantify the potential size of a seed bank by making the seeds able to germinate in unlimited light and moisture conditions. However, in natural environments, optimal conditions for germination and growth of seedlings happen sporadically and thus only a few viable seeds have the opportunity to germinate (Panufnik-Medrzycka and Kwiatkowska-Falinska, 2014). The whole number of seedlings emerging in particular ecological conditions in the field informs on the realised size of the seed bank.

The realised seed bank can produce a new cohort of plants for the re-establishment of populations when environmental conditions are favourable (Erfanzadeh et al., 2013; Ooi, 2012; Parker and Kelly, 1989). Its role is particularly important in relation to two of the most

prominent threats on forest ecosystems, i.e. habitat fragmentation and climate change. Climate change, through changes in temperature and rainfall patterns, may indirectly, yet significantly impact the population size and dynamics of seed banking species by influencing the timing of germination, seed maturation, seed mass and seed persistence in the soil (Walck et al., 2011). Forest fragmentation, may impact, for instance, seed bank composition and diversity (Lin et al., 2006), leading to a progressive reduction of typical forest species (Cadenasso and Pickett, 2001; Lin et al., 2006; Olden et al., 2004; Rooney et al., 2004). This trend can have detrimental consequences on species diversity, as the replacement of plant specialists by generalists leads to functional homogenization (Büchi and Vuilleumier, 2014; Clavel et al., 2011; Olden et al., 2004).

We know very little about the responses of forest species in terms of germination behaviour in the face of these environmental changes (Walck et al., 2011). Upland forest plants are of particular concern as these species are more range-restricted and likely to suffer more or go extinct with climate change (Parmesan, 2006). Although forest edge effects have been well studied over the past decade, the responses of the realised forest seed bank to microclimate are still poorly understood. Despite potential stronger edge effects in regions with warmer climates (Aussenac, 2000; De Frenne et al., 2019; Lembrechts and Lenoir, 2020), effects on southern European forests remain underexplored. Moreover, most seed bank studies focus on the potential seed bank, meaning to account for all viable seeds in the soil (Bossuyt and Honnay, 2008), regardless of microclimate effects. However, potential and realised seed banks can differ considerably, as shown, for example, by the decrease in light-demanding species found in the seed bank realised in natural forest conditions compared with that in a greenhouse (Panufnik-Medrzycka and Kwiatkowska-Falinska, 2014).

We surveyed the seed bank (transient and persistent seed bank) and studied edge effects on the germination responses from the seed bank of two types of European temperate deciduous forests, mesophilous forests of the Oceanic climatic region and thermophilous forests of the Mediterranean climatic region. We particularly focused on seedling responses (herbaceous and woody species) as these are understudied so far, but also expected to be more sensitive to environmental changes than the adult stages (Dalgleish et al., 2010; Fay and Schultz, 2009; Lloret et al., 2004). Accordingly, our research questions were:

- i) How do the forest edge and interior (realised) soil seed bank differ from each other in terms of seedling density, diversity and composition? (i.e. refers to edge vs. interior seed bank in their original, non translocated position).
- ii) Do edge effects cause changes in the seedling community that develop from the seed bank of the forest interior, and vice-versa? (i.e. refers to soil translocated from the forest edge to the interior and from the interior to the edge).
- iii) Does this effect change depending on latitude, elevation and forest structure?
- iv) Does climate warming influence seed bank regeneration of upland forest understorey species?

## 2. Materials and methods

### 2.1. Study area

We set up an experiment in two European regions representative of Mediterranean (Central Italy, 42° N) and Oceanic (Belgium, 50°

N) climate types, respectively, Koppen-climate-classification in Beck et al. (2018). The study was replicated at two elevations per region: high (312–798 m) and low (21–140 m) to account for the climatic variation associated with elevation. For the sake of brevity, we define the high elevation forests as uplands and the low elevation forests as lowlands. Moreover, the effect of forest structure on seed bank germination responses was evaluated by including two stand types, dense and open (Table A1). Dense forests are vertically complex forests, with multiple layers of trees and shrubs of different ages, with high canopy cover (trees + shrubs >120%), high basal area (between 19 and 52 m<sup>2</sup>/ha<sup>-1</sup>) and a well-developed shrub layer. Open forests have lower canopy cover (trees + shrubs <100%) and basal area (8–24 m<sup>2</sup>/ha<sup>-1</sup>) with a sparse shrub layer. Thus, in each region, four sites (Fig. 1) were selected with always two forest structural types at both elevations. The selected forests were mesophilous (Belgium) and thermophilous (Italy) deciduous forests dominated by oak (*Quercus* sp.) species. A detailed site description can be found in Table A1. All the edges were bordered by grassland or arable land and were south facing (Table A3).

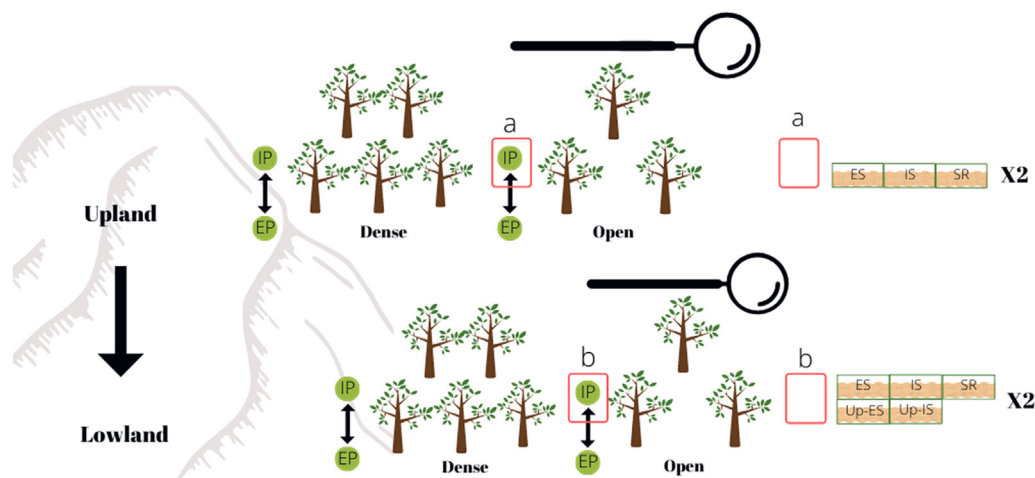
## 2.2. Experimental design

In each region we set up eight experimental plots (Fig. 1): two elevation belts (high and low elevation), two forest structural types (dense and open) and two positions (edge and interior), resulting in a total of 16 experimental plots (eight in Italy and eight in Belgium). Between January and February 2020, soil samples were collected in two quadrats (Q1 and Q2) at a distance of 0–2 m from the outermost line of tree trunks (forest edge), and in two quadrats at 98–100 m perpendicularly from the forest edge in the forest interior. The two quadrats were ca. 3.5 m from each other, and each was 2 m × 2 m, an area considered optimal for seed bank sampling (Plue et al., 2012). Soil samples collected within each quadrat were mixed and divided into three portions in the lowland sites and five portions in the upland sites. Each portion was spread on the top of one box prepared as below (par. 2.3). All boxes (96) always contained the same proportion of soil (ca. 0.9 L). For the low elevation sites two boxes (as two replicates) always remained in place, two were moved from the forest edge to the forest interior (and viceversa) and two were brought to the greenhouse. For the upland forest, two more boxes for each position (edge/interior) and forest type (dense and open) were brought to the lowland forest and placed in both edge and interior of the corresponding forest type. We thus applied a multi-factorial design with a total of 48 treatments and with two replicates of each treatment (96 boxes in total).

Soil translocation from upland to lowland areas simulated an artificial warming of 2.1–3.4 °C (soil) and 2.9–3 °C (air) for Italy, and 1.1–2.1 °C (soil) and 1–1.6 °C (air) for Belgium. These values were calculated as the difference of the mean soil and air temperature from upland minus lowland, and in the dense and open forests, respectively. Values correspond approximately to the predicted land temperature increase by the end of 2100, that is between 2.2 and 4 °C for the Mediterranean area (Cramer et al., 2018), and, in the best scenario (RCP4.5), between 1 and 4.5 °C for central Europe (Jacob et al., 2014). Air and soil temperature data were collected from microclimatic sensors placed next to the experimental plots (Meeussen et al., in press). Temperatures were recorded at hourly intervals (2018–2020) using a Lascar temperature logger (EasyLog EL-USB-1, accuracy at –35 to +80 °C: ± 0.5 °C). Air loggers were protected by a plastic shield and positioned at 1 m height. Soil loggers were buried in the ground in a protective plastic tube at a depth of 5 cm.

## 2.3. Soil sample collection

Within each quadrat, we randomly collected 75 and 45 soil samples, for the high and the low elevation, respectively. The soil samples were 5 cm deep × 4 cm diameter (litter removed) resulting in a soil volume of 9.3 and 5.6 L per plot for the upland and lowland plots, respectively. Soil samples were pooled together per quadrat, sieved with a 4-mm sieve and spread out in a 30 cm × 20 cm plastic box on top of a 15 cm deep layer of sterilized potting soil such that the forest soil layer was ca. 1 cm thick. Each box had holes at the bottom and a layer of expanded clay to allow drainage. At each location (edge and interior), one experimental plot was subsequently installed between the two quadrats. There, the boxes with the sieved seed bank samples were placed at the edge and forest interior in exchanged positions, as shown in Fig. 1. A control box, with a proportion of soil from each plot (ca. 0.9 L) was installed in a greenhouse with a natural sunlight regime mainly to facilitate the species identification of the seedlings. Boxes were buried in the forest soil such that the top was level with the soil surface. A plastic ‘root cloth’ was placed underneath to prevent direct contact with the forest soil and possible contamination with seed or vegetative parts (rhizome fragments, bulbils, parts of tubers, etc.) of other plant species in the forest soil. A thin mesh was also installed over the boxes at ca. 75 cm above the soil surface to prevent seed rain contamination. In addition, a natural ‘seed rain’ control box with sterilized potting soil but without added forest soil was left outside of the mesh. All plots were surrounded by a fence against disturbance by animals.



**Fig. 1.** Experimental design within a region: eight experimental plots (P) in total (four edge/interior sites). E = edge; I = interior; P = plot; S = soil. EP represents the forest edge plot, IP the forest interior plot. a) example of an experimental plot in the upland b) example of an experimental plot in the lowland. ES: soil from edge position, IS: soil from interior position, SR: natural seed rain, Up-ES: soil from upland edge position, Up-IS: soil from upland interior position. Arrows show the translocation of soil in the forest edge and interior and from up to lowland. This set-up was replicated twice and installed in Belgium and in Italy resulting in a total of 48 treatments and with two replicates of each treatment ( $n = 96$  boxes in total - excluding natural ‘seed rain’ boxes).

## 2.4. Field surveys

Two measurements in the experimental plots were done in April and July 2020. Firstly, each seedling emerging from the soil was recorded and identified up to the species level and mapped on a grid with 50 × 75 mm meshes laid down on the soil surface in the boxes (Fig. A2). This allowed to measure the same individuals of each species in the first and second survey and to determine mortality in July as the number of missing or dead individuals compared with the April survey. Secondly, ground cover was estimated for each individual as percentage of cover on one mesh of the grid. In July all surviving individuals were collected (shoots and roots) and biomass oven-dried at 40 °C for 48 h. The shoot was separated from the root and separately weighed to determine shoot and root biomass and ratios. Species nomenclature follows Euro+Med (2006).

## 2.5. Data analysis

To quantify the realised soil seed bank, we calculated the seedling density per square meter in each box considering all germinated seedlings separately for April and July surveys. Next, species diversity was quantified as species richness (richness), Shannon index (shannon, Formulae A1) and species Evenness (evenness, Formulae A2) of the seedling community in each box, in both surveys. The percentage of cover of the seedling community was visually estimated in each box. Biomass was determined as the sum of all individuals' biomasses (shoot and roots separately) in the seedling community in July. All these response variables were calculated for each of the 48 treatments. For statistical analyses the dataset was subdivided into three subsets: 1) edge soil in edge position vs. interior soil in interior position (32 observations), 2) edge soil in edge position vs. edge soil in interior position (48 observations), 3) interior soil in interior position vs. interior soil in edge position (48 observations). The first set of models (Table 1) tested the influence of the edge vs. interior position, region, forest type and elevation on the seed bank and seedling community. In these models, we considered the original edge and interior positions of the soil samples without any position exchange. With the second set of models, we tested the effects of 1) positioning of the forest interior seed bank at the forest edge; 2) translocation of forest interior soil from upland to lowland forest, in both interior and edge position. The third set of models analysed the effects of: 1) positioning of forest edge seed bank in the forest interior; 2) translocation of forest edge soil from upland to lowland forest, in both interior and edge position.

Generalized linear mixed-effects models were applied using the packages 'lme4' (Bates et al., 2015) and 'MuMIn' (Barton, 2019). Poisson distribution was used for the count data (species richness and seedling density), while a binomial distribution was adopted for the proportion

of generalists and forest specialists. To account for the nested experimental design and to avoid pseudo-replication caused by the irregular spatial distribution of the sites, a random intercept term with 8 levels, 'site', was used in all models (Fig. 1). The fixed structure of the model was composed of the four design variables (i.e. soil position, region, forest type and elevation) including all two-way interactions containing the soil position variable. Two-way interactions were chosen for sake of simplicity and based on our research questions. All continuous variables were standardized (z-transformation) to compare model coefficients. The following equation represents the starting model:

$$\begin{aligned} & \text{Variables} \sim \text{soil position} + \text{region} + \text{forest type} + \text{elevation} + \text{region} \\ & \quad \times \text{soil position} + \text{elevation} \times \text{soil position} + \text{forest type} \\ & \quad \times \text{soil position} + (1 | \text{site}) \end{aligned}$$

The starting model was the same for the three subsets of data. Only the variable "elevation" changed in the second and third data subsets to account for the translocation of soil from the upland to the lowland forest. The single best model was selected based on lower Akaike information criterion (AIC) and Bayesian information criterion (BIC) with the dredge-function of package MuMIn (Barton, 2019). Data exploration and model evaluation followed the protocol of Zuur et al. (2010). In all models, residuals were evaluated for normality and homogeneity by a visual check of the model assumptions (normality of residuals, normality of random effects, homogeneity of variance, multicollinearity). When overdispersion occurred in count data, we proceeded with a quasi-likelihood analysis to adjust the coefficient table by multiplying the standard error with the square root of the dispersion factor and recomputing the z- and p-values accordingly (Lee and Nelder, 2000).

Differences in community composition among regions were quantified with the *vegdist*-function of the R package 'Vegan v2.4-7' (Oksanen et al., 2020) using the Raup-Crick distance and visualized with nonmetric multidimensional scaling (NMDS) (*metaMDS*-function in *vegan*). Differences were tested using PERMANOVA (*adonis*-function in *vegan*; 999 permutations with strata = region) (Anderson, 2001). Multivariate homogeneity of dispersion was also tested using *betadisper* (*vegan*), restricting permutations by region. Also, Raup-Crick distances were used to quantify the average community composition between treatments (Edge & interior, Edge & edge in interior, Interior & interior in edge, Upland & Up to lowland), within each region. Differences between treatments and regions were tested with Kruskal Wallis test with the *kruskal.test*-function of 'stats' package followed by a Dunn's posthoc test made with *dunn.test*-function in 'dunn.test' package.

Next, we analysed the effects on plant ecological groups and functional types. For this purpose, the recorded species were assigned to four forest guilds following the classification proposed by Heinken et al. (2019) for the temperate and boreal European regions: species

**Table 1**

Summary of the results of the first set of models testing the influence of the four independent variables (soil position, region, forest type, elevation) on the abundance and diversity of the soil seed bank and seedling community at the forest edge and the forest interior. Two levels categorical predictors are: Soil position (edge soil in edge position, interior soil in interior position), Region (Italy, Belgium), Forest type (dense and open forest), Elevation (up, low). Edge position, Belgium, dense forest and up elevation are used as reference categories, respectively. Values are parameter estimates of linear mixed models (Shannon index, evenness, cover, biomass, mortality) and generalized linear mixed models (seedling density, species richness, specialists and generalists). Asterisks denote the significance level (with  $p < 0.05^*$ ,  $p < 0.01^{**}$ ,  $p < 0.001^{***}$ ). Marginal  $R^2$  and conditional  $R^2$  (Nakagawa and Schielzeth, 2013) represent the proportion of variation explained respectively by fixed factors and both random and fixed factors. Positive or negative values denote the direction of the effect. For variables explanation see Table B4.

| Edges vs. interiors | Soil position | Region   | Forest type | Elevation | Region:<br>Soil position | Elevation:<br>Soil position | $R^2$ marginal | $R^2$ conditional |
|---------------------|---------------|----------|-------------|-----------|--------------------------|-----------------------------|----------------|-------------------|
| Seedling density    | -1.641**      | 1.798    |             | 0.038     | 1.901**                  | -0.682*                     | 0.85           | 0.99              |
| Richness            | 0.095         | 2.288*** |             | 0.457*    |                          | -0.681*                     | 0.91           | 0.91              |
| Shannon             | -0.209        | 1.850*** |             | 0.194     |                          |                             | 0.81           | 0.82              |
| Evenness            | -0.373        | 0.057    |             | 0.187     |                          |                             | 0.03           | 0.14              |
| Cover               | -0.181        | 1.143    | 0.112       | 0.965     |                          | -0.362                      | 0.39           | 0.52              |
| Biomass shoot       | -0.169        | 0.438    |             | -0.008    |                          |                             | 0.05           | 0.11              |
| Biomass root        |               | 0.138    | 0.714       | -0.282    |                          |                             | 0.15           | 0.19              |
| Mortality           | 1.033**       | -0.744   |             |           | -0.940                   |                             | 0.49           | 0.49              |
| Specialists         | 0.191         |          | -0.580*     | -1.445*** |                          |                             | 0.17           | 0.18              |
| Generalists         | -0.748*       |          | 0.384       | 0.667     |                          | 1.339*                      | 0.16           | 0.16              |

which can be mainly found in closed forests (1.1); species which occur typically along forest edges and in forest openings (1.2); species occurring in both forest and open vegetation (2.1); species occurring partly in forests, mainly in open vegetation (2.2) (Table A4); true “open habitat” species (O) were not observed in our study. A few Mediterranean species were not included in Heinken et al. (2019). These were classified based on Pignatti et al. (2017–2019) and our expert evaluation. For the purpose of statistical analyses, species were grouped in only two categories, forest specialists (guilds 1.1 + 1.2) and generalists (guilds 2.1 + 2.2). Seedlings only determined to the genus level were excluded from this analysis (4 individuals), as well as 13 unidentified species (42 individuals).

For the functional type analysis, each seed bank species was assigned to one of the following functional types: annual herbs (therophyte), perennial herbs (hemicryptophyte), graminoid (annual or perennial), geophyte, woody species (tree or shrub). Due to non-normal distribution of the data, differences between groups were analysed with non-parametric Kruskal Wallis test with the *kruskal.test*-function of ‘stats’ package. A Dunn’s posthoc test was made with *dunn.test*-function in ‘dunn.test’ package.

Finally, we compared the ecological profiles of the seedling communities using community weighted means of the species with Ellenberg indicator values: light, moisture and temperature (Ellenberg et al., 1991; Pignatti et al., 2005). Differences between groups (combination of type of soil and position) were tested with one-way ANOVA with the *aov*-function of the ‘stats’ package followed by a posthoc Tukey’s HSD test with the ‘*TukeyHSD*’ of package ‘stats’. The hypothesis of normality of distribution was tested with Lilliefors (Kolmogorov-Smirnov) test with the function *lillie.test* in package ‘nortest’. Homogeneity of variances was tested with Bartlett’s test with the function *bartlett.test* in package ‘stats’. Letter summary of statistical similarities and differences was visualized with *multcompLetters* function of ‘multcompView’ package. All data analyses were performed in R-4.0.5 (R Core Team, 2019).

### 3. Results

In total, 1786 seedlings, belonging to 145 species (Table B5), emerged from the seed bank: 113 species were found in Italy and 19 in Belgium, while 13 were detected in both regions; four seedlings could be identified only at genus level and 13 species remained unidentified (42 individuals). The most abundant species in the seed bank were *Campanula rapunculus* (103 seedlings), *Moehringia trinervia* (92), *Erigeron canadensis* (71), *Prunella vulgaris* (68), *Inula conyzae* (64), *Clinopodium vulgare* (62), *Luzula forsteri* (58) and *Rubus fruticosus* (54). Mean and standard deviation values of the analysed variables in each region and soil type/position are given in Table B6, separately for the two surveys (April and July). A total of 492 seedlings, belonging to 11 species, were forest specialists. The mortality rate between April and July was high for *Betula pendula* (7/14), *Callitriche stagnalis* (13/13), *Carpinus betulus* (16/25), *Gnaphalium uliginosum* (13/14), *Moehringia trinervia* (58/93), *Poa trivialis* (21/26), *Rubus fruticosus* (27/54), *Silene gallica* (9/9) and *Urtica dioica* (32/45).

Overall, substantial differences were detected between regions and between both soils in their original (edge vs. interior) and exchanged position (edge to interior and vice versa). Generally, the seed bank at the forest edge was significantly different from that at the forest interior in terms of seedling density and diversity (Shannon index). However, patterns of variation were not consistent between regions. Moreover, species richness, Shannon index and April evenness were considerably higher in Italy. Generally, lowland forests held more richness compared to upland forests but with a lower proportion of forest specialists.

#### 3.1. Seedling density

We found significant interactive effects between the forest interior and region in terms of seedling density ( $p = 0.002$ ): seedling density at the forest interior was higher than at the forest edge in Italy but

lower in Belgium (Table 1). Also, a negative interaction between interior position and low elevation was found ( $p = 0.02$ ), indicating a lower seedling density in interior vs. edge position in lowland forests. In the interior soil moved to the edge (Table 2) we found the same contrasting pattern: the movement of soil from the interior to the edge positively influenced the seedling density in Belgium but negatively in Italy ( $p = 0.01$  in both cases). Concerning the edge soil, moving it to the interior had a negative influence on seedling density in Belgium but positive in Italy (Table 3). Translocation of edge soil from upland to lowland (Table 3) resulted in a decrease of seedling density ( $p = 0.007$ ), while the interior soil was not affected (Table 2). Finally, moving the interior soil to the edge resulted in higher seedling density in Italy, but not in Belgium (Table 2).

#### 3.2. Diversity

In non-moved soil, the species richness of the seedling community was significantly higher in Italy compared to Belgium ( $p < 0.0001$ ) and at low vs. high elevation ( $p < 0.0001$ ) (Table 1). Species richness was particularly lower in the interior than at the edge of lowland forests as shown by the negative interaction between soil position and elevation ( $p = 0.04$ ; Table 1). The best model for Shannon index showed higher values ( $p = 0.03$ ) at the forest interior and at low elevation ( $p = 0.03$ ) (Table B1). Shannon index and evenness were always higher in Italy ( $p < 0.0001$ ,  $p = 0.05$ ) compared to Belgium (Table 1 and B1). In moved soil (Tables 2 and 3), differences between Italy and Belgium in terms of species richness (both  $p < 0.0001$ ) and Shannon ( $p < 0.001$ ,  $p < 0.0001$ ) were highly significant. In interior soil moved to the edge (Table 2), the Shannon index was higher compared to non-moved soil ( $p = 0.02$ ; Table 2). The translocation of edge soil from upland to lowland had a negative influence on species richness ( $p = 0.02$ ; Table 3).

#### 3.3. Species composition

The seed-bank in Italian and Belgian soil samples was compositionally very different (Fig. 2). In Belgium, a more homogeneous seedling community was found at the forest edge and interior compared to Italy. The difference between Belgium and Italy in the mean compositional Raup-Crick distance between edges and interiors was in fact significant (Belgium:  $0.16 \pm 0.19$ ; Italy:  $0.54 \pm 0.18$ ;  $p = 0.02$ ). Similarly, the mean Raup-Crick distance between edge soil in edge and interior position was  $0.10 \pm 0.11$  for Belgium and  $0.41 \pm 0.15$  for Italy ( $p = 0.04$ ). Also, moving the soil from upland to lowland forest resulted in larger compositional differences of the seedling community in Italy; the mean Raup-Crick distance was  $0.17 \pm 0.24$  and  $0.56 \pm 0.11$  for Belgium and Italy, respectively ( $p = 0.01$ ).

#### 3.4. Cover and biomass

Overall, only in a few cases the total cover and biomass of the seedling community were affected by position (edge vs. interior), forest type and elevation. However, for the April cover, a significant interaction effect was found between soil position and forest type ( $p = 0.01$ ), with the cover being higher at the interior of open forests (Table B1) compared to the interior of dense forests. In moved soil (both directions-Tables 2 and 3), the total cover was higher in Italy compared to Belgium ( $p = 0.01$ ,  $p = 0.03$ ). Only the root biomass was affected by position exchange (edge soil in interior position) being lower after movement to the interior ( $p = 0.03$ ; Table 3).

#### 3.5. Mortality

The mortality rate was highest at the forest interior ( $p = 0.008$ ; Table 1). In interior soil moved to the edge the mortality rate was lower in Italy ( $p < 0.0001$ ) and after movement of soil from high to low elevation ( $p = 0.04$ ; Table 2). In edge soil moved to the interior,

**Table 2**

Summary of the results of the second set of models testing the influence of the four independent variables (soil position, region, forest type, elevation) on the abundance and diversity of the seed bank and seedling community at the forest interior compared to that of the interior moved to the edge. Categorical predictors are: Soil position (2 levels: interior soil in interior position, interior soil in edge position), Region (2 levels: Italy, Belgium), Forest type (2 levels: dense and open forest), Elevation (3 levels: up, low, up to low). Interior soil in interior position, Belgium and dense forest are used as reference categories for soil position, region and forest type, respectively. Up elevation is used as reference category for both Up to low elevation and Low elevation. Values are parameter estimates of linear mixed models (Shannon index, evenness, cover, biomass, mortality) and generalized linear mixed models (seedling density, species richness, specialists and generalists). Asterisks denote significance level (with  $p < 0.05^*$   $p < 0.01^{**}$   $p < 0.001^{***}$ ). Marginal  $R^2$  and conditional  $R^2$  (Nakagawa and Schielzeth, 2013) represent the proportion of variation explained respectively by fixed factors and both random and fixed factors. Positive or negative values denote the direction of the effect. For variables explanation see Table B4.

| Interior vs. interior in edge | Soil position | Region    | Forest type | Up to low elevation | Low elevation | Region: Soil position | Up to low elevation: Soil position | Elevation: Soil position | R <sup>2</sup> marginal | R <sup>2</sup> conditional |
|-------------------------------|---------------|-----------|-------------|---------------------|---------------|-----------------------|------------------------------------|--------------------------|-------------------------|----------------------------|
| Seedling density              | 1.076*        | 3.420**   |             | -0.112              | 0.044         | -1.063*               | -0.107                             | 0.043                    | 0.96                    | 0.99                       |
| Richness                      | 0.754         | 2.889***  |             | -0.035              | -0.065        | -0.575                |                                    |                          | 0.91                    | 0.93                       |
| Shannon                       | 0.191*        | 1.877***  |             | -0.091              | 0.073         |                       |                                    |                          | 0.90                    | 0.92                       |
| Evenness                      | 0.179         | 0.504     | 0.507       |                     |               |                       |                                    |                          | 0.15                    | 0.27                       |
| Cover                         | 0.120         | 1.412*    | 0.408       | 0.403               | 0.324         |                       |                                    |                          | 0.61                    | 0.63                       |
| Biomass shoot                 | -0.121        | 0.701     | -0.037      |                     |               |                       |                                    |                          | 0.12                    | 0.13                       |
| Biomass root                  | -0.035        | 0.457     | 0.269       |                     |               |                       |                                    |                          | 0.06                    | 0.11                       |
| Mortality                     | -0.162        | -1.170*** |             | -0.566*             | -0.062        |                       |                                    |                          | 0.43                    | 0.43                       |
| Specialists                   |               |           | -0.138      | -0.389              | -1.693***     |                       |                                    |                          | 0.14                    | 0.14                       |
| Generalists                   | -0.185        |           |             | 0.308               | 1.591***      |                       |                                    |                          | 0.13                    | 0.13                       |

mortality increased in Belgium ( $p < 0.0001$ ) but decreased in Italy ( $p = 0.01$ ; Table 3). The mortality rate of seedlings from edge soil was generally lower in Italy compared to Belgium ( $p = 0.002$ ; Table 3).

### 3.6. Forest specialists and generalists

The proportion of forest specialist taxa varied with forest type and elevation, being lower in open forests ( $p = 0.04$ ) and lowland forests ( $p < 0.0001$ ; Table 1). The proportion of generalists was influenced by edge/interior position and decreased from edge to interior in upland forests ( $p = 0.04$ , Table 1), whereas it increased in lowland forests ( $p = 0.02$ ; Table 1). The proportion of specialists and generalists in interior soil moved to the edge (Table 2) was only influenced by elevation: the number of specialists was significantly lower in lowland forests ( $p < 0.0001$ ), while the number of generalists was higher ( $p < 0.0001$ ). The proportion of specialists in the edge soil (Table 3) decreased after translocation from upland to lowland forest ( $p = 0.01$ ), while the proportion of generalists increased ( $p = 0.003$ ). The proportion of both specialist and generalist taxa in edge soil was significantly higher in lowland forests ( $p = 0.02$ ,  $p = 0.04$ ; Table 3).

### 3.7. Functional types and Ellenberg indicator values

Overall, edge and interior seedling communities differed in the cover of geophytes ( $p = 0.01$ ) and graminoids ( $p = 0.004$ ; Table 4). The

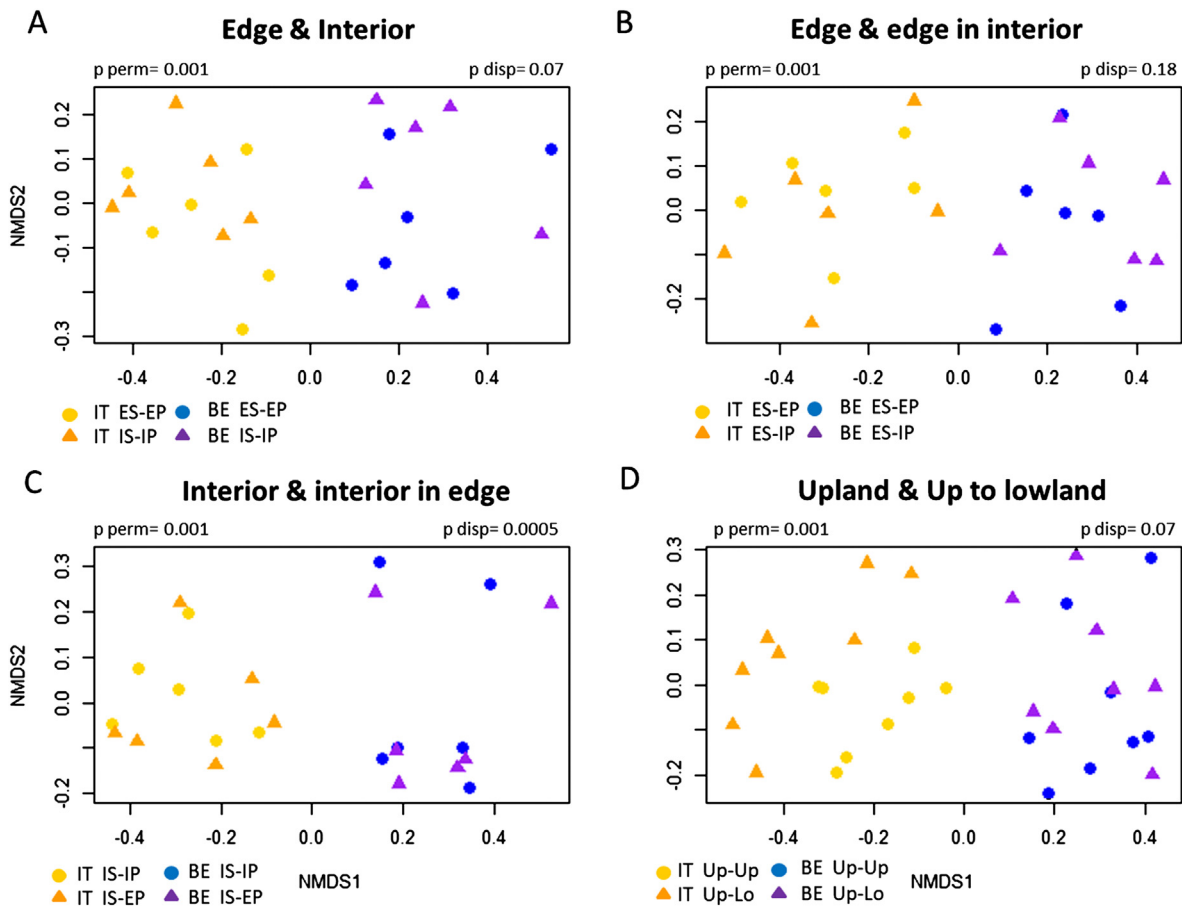
movement of soil from the interior to the edge position resulted in a higher cover of shrubs and trees ( $p < 0.05$ ). No significant differences after soil position exchange were detected for the other functional groups. The edge seedling community differed from that moved to the interior in terms of perennial herbs ( $p < 0.001$ ), annuals ( $p < 0.001$ ), graminoids ( $p = 0.01$ ) and shrubs and trees ( $p < 0.001$ ), since the cover of all these plant functional types decreased significantly after movement (Table 4). The seed bank of upland forests moved to lowland produced a seedling community with a higher cover of graminoids ( $p < 0.0001$ ), perennial herbs ( $p < 0.0001$ ) and annuals ( $p < 0.001$ ; Table 4).

Concerning Ellenberg indicator values, in the Belgian sites no differences were detected in light, moisture and temperature between the seedling communities at the edge and interior, neither before nor after position exchange (Fig. 3). The seedlings developed in the interior seed bank of the Italian soil samples were instead, significantly less light-demanding compared to the edge seedlings ( $p = 0.04$ ; Fig. 3) while after movement to the edge, higher-light demanding ( $p = 0.03$ , Fig. 3). The edge and interior seedling communities in Italy differed also in moisture requirements, which was higher at the forest interior ( $p = 0.04$ ). Moving the upland forest soils to lowland sites resulted in seedling communities with higher mean light values, in both study regions (Belgium  $p = 0.001$ , Italy  $p = 0.01$ ; Fig. 4). Furthermore, the seedling community was composed of more thermophilous species in Belgium ( $p = 0.02$ ) while no significant variations were detected in moisture and temperature indicator values in Italy (Fig. 4).

**Table 3**

Summary of the results of the third set of models testing the influence of the four independent variables (soil position, region, forest type, elevation) on the abundance and diversity of the seed bank and seedling community at the forest edge compared to that of the edge moved in the interior. Categorical predictors are: Soil position (2 levels: edge soil in edge position, edge soil in interior position), Region (2 levels: Italy, Belgium), Forest type (2 levels: dense and open forest), Elevation (3 levels: up, low, up to low). Edge soil in edge position, Belgium and dense forest are used as reference categories for soil position, region and forest type, respectively. Up elevation is used as reference category for both Up to low elevation and Low elevation. Values are parameter estimates of linear mixed models (Shannon index, evenness, cover, biomass, mortality) and generalized linear mixed models (seedling density, species richness, specialists and generalists). Asterisks denote significance level (with  $p < 0.05^*$   $p < 0.01^{**}$   $p < 0.001^{***}$ ). Marginal  $R^2$  and conditional  $R^2$  (Nakagawa and Schielzeth, 2013) represent the proportion of variation explained respectively by fixed factors and both random and fixed factors. Positive or negative values denote the direction of the effect. For variables explanation see Table B4.

| Edge vs. edge in interior | Soil position | Region   | Forest type | Up to low elevation | Low elevation | Region: Soil position | Up to low elevation: Soil position | Low elevation: Soil position | R <sup>2</sup> marginal | R <sup>2</sup> conditional |
|---------------------------|---------------|----------|-------------|---------------------|---------------|-----------------------|------------------------------------|------------------------------|-------------------------|----------------------------|
| Seedling density          | -0.846*       | 2.043    |             | -0.548**            | -0.287        | 0.890*                |                                    |                              | 0.75                    | 0.99                       |
| Richness                  |               | 2.155*** |             | -0.335*             | 0.194         |                       |                                    |                              | 0.89                    | 0.91                       |
| Shannon                   | -0.098        | 1.832**  | 0.216       | -0.186              | 0.186         |                       |                                    |                              | 0.72                    | 0.85                       |
| Evenness                  | -0.422        | 0.267    |             |                     |               |                       |                                    |                              | 0.52                    | 0.52                       |
| Cover                     | -0.311        | 1.406*   | 0.141       | 0.448               | 0.965         |                       | 0.324                              | -0.071                       | 0.49                    | 0.68                       |
| Biomass shoot             | -0.439        | 0.679    | 0.436       |                     |               |                       |                                    |                              | 0.19                    | 0.38                       |
| Biomass root              | -0.499*       | 0.557    | 0.674       |                     |               |                       |                                    |                              | 0.24                    | 0.38                       |
| Mortality                 | 1.035***      | -0.908** |             |                     |               | -0.992*               |                                    |                              | 0.58                    | 0.58                       |
| Specialists               |               |          | -0.501      | -0.780*             | 1.080*        |                       |                                    |                              | 0.07                    | 0.11                       |
| Generalists               | -0.284        |          | 0.166       | 0.958**             | 0.937**       |                       |                                    |                              | 0.06                    | 0.11                       |



**Fig. 2.** Non metric multidimensional scaling (NMDS) scatterplots showing species composition differences between regions (IT = Italy and BE = Belgium) in the seedling communities developed under the various treatments: Edge & interior = edge soil in edge position vs. interior soil in interior position; Edge & edge in interior = edge soil in edge position vs. edge soil in interior position, Interior & interior in edge = Interior soil in interior position vs. interior soil in edge position, Upland & Up to lowland = upland soil in upland forests vs. upland soil in lowland forests. E = edge; I = interior; P = plot; S = soil.

**4. Discussion**

**4.1. Seedling density, composition and mortality**

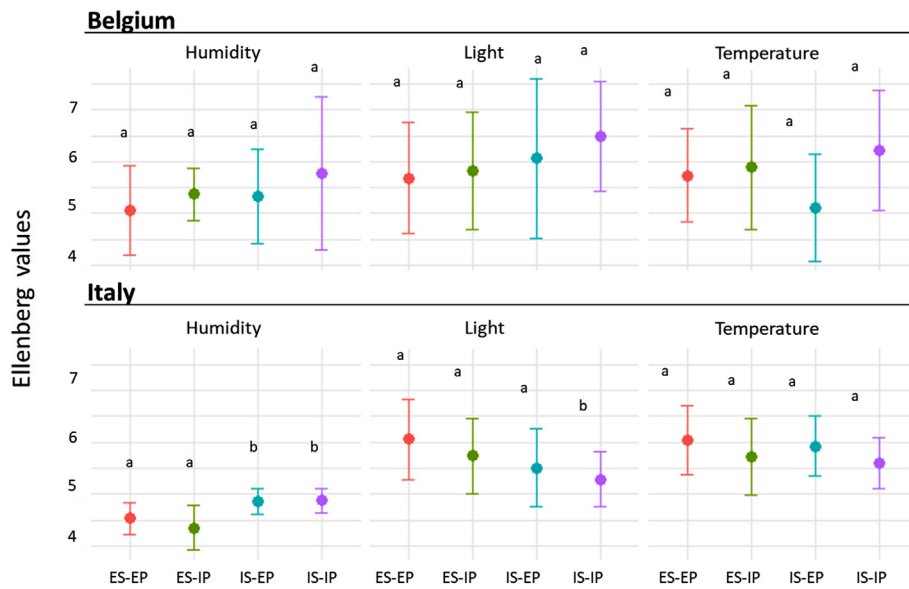
Variation in seedling density from the seed bank of soils at forest edges vs. interiors was considerable. First, seedling density was generally higher at the edge of mesophilous Belgian forests (vs. interior) but lower at the edge of Italian thermophilous forests compared to forest interiors. Results for Belgium seems to support previous findings by Cadenasso and Pickett (2001), Devlaeminck et al. (2005) and Koncz et al. (2011) showing that seed bank density significantly decreases

from the edge to the interior of the forest. These studies, however, were finalized to determine all the seeds present in the soil in standard conditions (potential seed bank), without taking into account the possible effects of microclimate on their germination (realised seed bank). Lack of investigations on seed-bank of thermophilous deciduous forests in southern Europe does not allow us to compare our findings for this forest type, although these seem more in line with other studies reporting no decrease in seed abundance with increasing distance from the edge to the forest interior (Lin et al., 2006; Lin and Cao, 2009). For Italy, the forest interior conditions, i.e. a more stable microclimate and lower soil temperature compared to the edge

**Table 4**

Mean  $\pm$  standard deviation of cover values of five main plant functional groups arranged by soil type and position (ES-EP = edge soil in edge position, IS-IP = interior soil in interior position, IS-EP = interior soil in edge position, ES-IP = edge soil in interior position) and elevation (Upland = upland soil in upland position; Up to lowland = upland soil in lowland position, Lowland = lowland soil in lowland position). Superscript letters indicate statistically different groups at  $p < 0.05$ .

|                                    | ES-EP                         | IS-IP                          | IS-EP                         | ES-IP                        | p value    |
|------------------------------------|-------------------------------|--------------------------------|-------------------------------|------------------------------|------------|
| Geophytes                          | 3.92 $\pm$ 5.39 <sup>a</sup>  | 0.33 $\pm$ 0.58 <sup>b</sup>   | 0.03 $\pm$ 0.04 <sup>b</sup>  | 2.42 $\pm$ 1.70 <sup>a</sup> | p = 0.001  |
| Annual herbs                       | 5.5 $\pm$ 7.55 <sup>a</sup>   | 6.53 $\pm$ 12.45 <sup>ac</sup> | 4.02 $\pm$ 7.36 <sup>bc</sup> | 3.26 $\pm$ 6.20 <sup>b</sup> | p = 0.007  |
| Perennial herbs (Hemycryptophytes) | 4.74 $\pm$ 10.07 <sup>a</sup> | 4.6 $\pm$ 6.64 <sup>a</sup>    | 4.87 $\pm$ 6.65 <sup>a</sup>  | 2.26 $\pm$ 2.90 <sup>b</sup> | p < 0.0001 |
| Graminoids                         | 3.01 $\pm$ 5.03 <sup>a</sup>  | 1.26 $\pm$ 1.09 <sup>b</sup>   | 1.35 $\pm$ 1.62 <sup>b</sup>  | 2.62 $\pm$ 6.74 <sup>b</sup> | p = 0.02   |
| Woody species                      | 1.43 $\pm$ 1.65 <sup>ab</sup> | 1.82 $\pm$ 2.79 <sup>b</sup>   | 4.17 $\pm$ 8.40 <sup>a</sup>  | 0.77 $\pm$ 1.28 <sup>c</sup> | p < 0.0001 |
|                                    | Upland                        | Up to lowland                  | Lowland                       |                              | p value    |
| Geophytes                          | 3.01 $\pm$ 5.27               | 7.49 $\pm$ 13.86               | 1.79 $\pm$ 0.96               |                              | ns         |
| Annual herbs                       | 2.47 $\pm$ 5.69 <sup>a</sup>  | 9.66 $\pm$ 13.32 <sup>b</sup>  | 6.34 $\pm$ 9.72 <sup>c</sup>  |                              | p < 0.0001 |
| Perennial herbs (Hemycryptophytes) | 3.23 $\pm$ 5.26 <sup>a</sup>  | 9.52 $\pm$ 13.03 <sup>b</sup>  | 4.34 $\pm$ 8.01 <sup>b</sup>  |                              | p < 0.0001 |
| Graminoids                         | 1.42 $\pm$ 3.06 <sup>a</sup>  | 3.73 $\pm$ 5.50 <sup>b</sup>   | 2.32 $\pm$ 4.78 <sup>b</sup>  |                              | p < 0.0001 |
| Woody species                      | 2.12 $\pm$ 6.01               | 4.62 $\pm$ 9.19                | 2.29 $\pm$ 3.43               |                              | ns         |

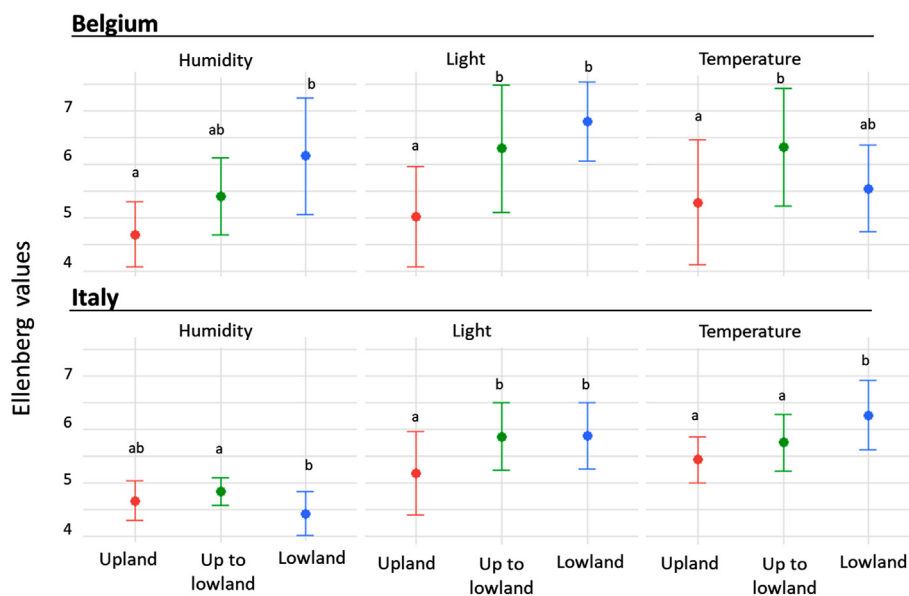


**Fig. 3.** Community weighted means of Ellenberg values with error bars for humidity, light and temperature of seedling communities from different soil types and position (ES-EP = edge soil in edge position, ES-IP = edge soil in interior position, IS-EP = interior soil in edge position, IS-IP = interior soil in interior position), separately by regions (Belgium and Italy). Letters indicate statistical differences between groups at  $p < 0.05$ .

(Davies-Colley et al., 2000; Matlack, 1993; Meeussen et al., in press; Schmidt et al., 2019), could have favoured the conservation of a higher proportion of seeds. Indeed, there is evidence that seed survival (Davis et al., 2005) and persistence (Pakeman et al., 1999) is lower in warmer conditions (i.e. forest edge).

The direction of the responses to soil position exchange also depended on climatic regions, with generally contrasting responses. Noteworthy, edge microclimate decreased seedling density of the interior seed bank in Italy, while it had a positive effect in Belgium. Conversely, the seedling density of the edge soil increased when transplanted to the interior in Italy, but decreased in Belgium. Hence, our findings provide evidence that edge microclimatic conditions can influence germination from the seed bank in contrasting ways, largely depending on macroclimate. Edge-to-interior microclimate gradients

in European forests significantly reduce temperature extremes (Davies-Colley et al., 2000; Matlack, 1993; Schmidt et al., 2019), especially in warmer regions (De Frenne et al., 2019; Meeussen et al., in press), which might also affect germination responses from the seed bank. At forest edges, increased exposure to solar radiation and wind rises the vapour pressure deficit (VPD; the difference between actual versus potential moisture content of the air, measured as atmospheric pressure) and reduces soil moisture (Reinmann et al., 2020; Ritter et al., 2005; Riutta et al., 2012; Smith et al., 2018). Moisture deficit may have negatively affected germination in warmer climates after translocation of soil to the edge position. Conversely, the overall positive influence of the interior position in warmer regions may indicate the beneficial role of the interior forest microclimate in reducing drought and heat (Matlack, 1993; Reinmann et al., 2020; Schmidt et al., 2019),



**Fig. 4.** Community weighted means of Ellenberg values with error bars for humidity, light and temperature of seedling communities from different elevations and in soil moved from high to low elevations (Upland = upland soil in upland position; Up to lowland = upland soil in lowland position; Lowland = lowland soil in lowland position), separately by regions (Belgium and Italy). Letters indicate statistical differences between groups at  $p < 0.05$ .



while in cooler climates this reduction may be less relevant (De Frenne et al., 2019; Meeussen et al., in press) and edges, at least for some plant groups, could provide more favourable conditions for germination than interiors (Matlack, 1993).

The magnitude of species composition differences between the edge and interior was different between the two regions, again supporting different functional seed bank responses to change of microclimatic conditions in mesophilous and thermophilous forests. In mesophilous forests, a more homogeneous seeding community consistently developed in all cases (edge vs. interiors, edge in edge position vs. edge in interior position, and vice versa), while in thermophilous forests different seedling communities developed depending on the microclimate to which soil samples were exposed. This was paralleled by significant differences between edge and interior in Ellenberg light and soil moisture values of the seedling communities in Italy, whereas no differences emerged in Belgium (Fig. 3). Regeneration by seed is driven by the co-occurrence of multiple ecological drivers that regulate seed dormancy (Walck et al., 2011) and each species has a set of environmental conditions under which it will germinate and grow. Hence, our findings may indicate that the seed bank of thermophilous forests of southern regions is more adaptable to the environment, due to the co-existence of numerous species that produce diverging seedling assemblages, depending on how variation in environmental conditions affects their germination. In our experiment, it is likely that a lower proportion of seeds of mesophilous species germinated after translocation from the interior to the edge due to lower moisture (Matlack, 1993; Riutta et al., 2012) and, conversely, that more edge seeds germinated after translocation to the interior thanks to higher air and soil moisture in the forest interior (Reinmann et al., 2020; Ritter et al., 2005; Smith et al., 2018). Hence, this soil translocation experiment suggests that moisture can act as a crucial limiting factor for seed banking species in southern regions. The consistently lower seedling mortality in the interior position in Italy further supported this assumption. On the contrary, mortality was higher at the interior and after change from edge to the interior in Belgium, possibly due to decreased light availability in combination with more light-demanding species being present in these edge seed banks. In fact, in the forest understorey, seed bank species usually emerge when light is not limiting (Naaf and Wulf, 2007; Plue et al., 2017b; Pykälä, 2004) and competition with shade-tolerant perennials is low (Godefroid et al., 2005). Conversely, canopy closure usually does not favour germinations and thus maintains or returns heliophilous species to the seed bank (Plue et al., 2010, 2017b) reducing their competition with the shade-tolerant forest species.

#### 4.2. Diversity

Overall, the species richness of the seedling community was not affected by edge vs. interior position neither in original position nor after position exchange, while Shannon diversity of interior soil possibly benefited from the edge microclimate due to improved light conditions. Our findings are not fully consistent with evidence from the few previous investigations of edge-to-interior species variations in the forest seed bank, which detected higher species richness at the forest edge (Devlaeminck et al., 2005; Yan et al., 2013). However, this difference could be due to differing methods since these investigations were greenhouse-based, hence meeting more favourable conditions for ruderal species but less so for habitat specialists with complex, habitat-specific germination requirements (Grime et al., 1981; Plue et al., 2017b; Ten Brink et al., 2013; Vandeloos et al., 2008).

#### 4.3. Generalists and specialists

As in the study of the understorey vegetation (Govaert et al., 2020), that included the same sites considered here, the proportion of generalist species was higher in the edge seed bank (non moved soil), and decreased in the forest interior, where it was always lower (non moved

soil). Forest structure had a clear influence on the proportion of germinated specialists and generalists: in the seed bank of open stands developed less specialists and more generalists than in dense stands. This could both mean that less specialists were present in the seed bank of open forest compared to dense forests, as it is in the understorey (Govaert et al., 2020) or that, with improved light conditions, more generalists germinated at the expense of specialists. However, the first hypothesis seems more likely and would support previous findings that dense forest edges act as barriers preventing or limiting the inflow of seeds of edges and openings that become incorporated in the forest seed bank (Cadenasso and Pickett, 2001; Devlaeminck et al., 2005; Didham and Lawton, 1999; Honnay et al., 2002). Indeed, forest structure did not influence the proportion of generalists found in the interior moved to the edge, corroborating this evidence.

A higher proportion of specialists was found in upland forests, as well as a higher proportion of generalists in lowland forests. This pattern could be linked to the usually more anthropogenic and fragmented forest landscapes at low elevations (Bertrand et al., 2011). Forest edge vs. interior microclimate after position exchange had a minor influence on the proportion of generalists and specialists in the short term. Non-forest species are mostly abundant in the seed bank (Bossuyt and Honnay, 2008; Decocq et al., 2004; Erenler et al., 2010; Koncz et al., 2011; Lin et al., 2006) but are not able to establish large populations in the stress imposing forest environment (Bossuyt and Hermy, 2001). Noteworthy, our findings support this evidence by the decreasing proportion of generalists in the edge soil moved to the interior. But what happens when a forest interior becomes an edge? Based on our findings, change of position from the interior to the edge influenced neither the proportion of specialists nor that of generalists that germinate from the seed bank. Since specialists are more shade-tolerant than generalists, we would have expected a higher proportion of generalists after movement from the interior to the edge, which was not the case. Specialists were able to germinate and establish in a warmer and brighter environment, whereas, conversely, generalists rapidly declined in the interior, likely due to a shortage of light. However, there is not sufficient evidence to say in the long period whether the specialists could eventually compete with the generalists in this new environment. This opportunity seems to be unlikely because when specialist species are maintained below their habitat carrying capacity (such as when environmental disturbances occur) generalist species are usually favoured (Brown, 1996; Büchi and Vuilleumier, 2014). However, when specialist interspecific competition is strong (i.e. high germination of specialists), generalist species could be gradually eliminated (Futuyma and Moreno, 1988).

#### 4.4. Ecological and functional types

Although relationships between disturbance, functional type and life-form patterns of plant communities have been widely described (Diaz and Cabido, 1997; Heinken and Weber, 2013; McIntyre et al., 1995), no studies have focused on edge effects on the functional composition of the seed bank. We found a higher proportion of geophytes and graminoids in the forest edge seed bank than in the interior. Moreover, edges positively influenced the abundance of seedlings of woody species germinating from the interior soil, while the interior position decreased the cover of all functional groups of the edge seed bank, except for geophytes. Trees and shrubs usually respond positively to higher light availability (Torrás et al., 2008). Similarly, annuals generally take advantage of disturbance associated with openings and higher light availability (McIntyre et al., 1995). In our study, annuals did not increase after movement from the interior to the edge but, instead, they decreased after soil movement from the edge to the interior. All functional types, except geophytes, showed an increased cover percentage after translocation of soil from upland to lowland forests, likely due to a stimulation of the growth rate driven by the higher temperatures. Generalists were favoured by warmer temperatures, while the response of most forest specialists was absent or weaker.

It is argued that shade-tolerant understorey herbs do not appear in forest seed banks, or are present at very low densities (Bossuyt and Hermy, 2001; Koncz et al., 2011; Mitlacher et al., 2002; Olano et al., 2002). However, this was recently imputed by a combination of a low surface sampling effort and poorly adapted greenhouse parameters during greenhouse experiments that may underestimate forest specialists presence (Plue et al., 2017a, 2012; Vandvik et al., 2016). In fact, the emergence of specialists in disturbance gaps implies that they are not exceptionally rare in the seed bank (Kalamees et al., 2012; Plue et al., 2021, 2012; Vandvik et al., 2016). In Italy, we found significant differences in the community weighted means of temperature and moisture (Pignatti et al., 2005) between the edge and the interior seedling communities, showing the latter to be formed by a high proportion of shade-tolerant species with high humidity requirements. Furthermore, the abundance of typical understorey species of mature forests such as *Brachypodium sylvaticum*, *Festuca heterophylla*, *Euphorbia amygdaloides*, *Moehringia trinervia*, *Poa trivialis* subsp. *sylvicola*, *Poa nemoralis* and *Viola reichenbachiana* (Heinken et al., 2019; Pignatti et al., 2017–2019), indicated that, for some shade-tolerant plants, seed banking is a feasible strategy to persist and spread in the forest community. Remarkably, the translocation of soil from the interior to the edge flattened these differences, favouring the development of more light-demanding communities.

#### 4.5. Effects of upland to lowland translocation experiment

Temperature influences germination rate and seedling establishment, suggesting that warming will affect, in the long term, the composition of the aboveground vegetation and, in turn, the future seed bank of forest communities. Moreover, seedling emergence is usually synchronized with seasonal changes in the environment (Baskin and Baskin, 2014; Fenner and Thompson, 2005; Walck et al., 2011) implying that forecasted changes in ecological cues may preclude, delay, or enhance regeneration from seeds, depending on the species (Walck et al., 2011). In our experiment, the translocation of soil from upland (between 300 and 800 m) to lowland areas (between 0 and 150 m) negatively affected seedling density, in both interior and edge soil. An accelerated decline of seed viability by increased soil temperatures was observed by Ooi (2012), and depletion of seed banks in response to changing rainfall patterns driven by climate change was reported by Basto et al. (2018). Lower seedling density in our experiment was likely caused by the less optimal conditions for germination, including changed rainfall and relative air humidity patterns at lowland sites. Interestingly, translocation of soil to lowland did not affect the diversity of the resulting seedling communities, except for a single case of decreasing species richness in edge soil. Translocation to lowland sites influenced the relative abundance of the main ecological groups: while forest specialists decreased, generalist species significantly increased likely due to the warmer climate, leading to a generally increased cover (Egli et al., 2004; Rustad et al., 2001; Buermann et al., 2018) of the seedling communities at lowland sites. Seed germination requirements of forest specialists were therefore not fully met (i.e. absence of chilling), while generalists found suitable conditions to germinate and even increased their growth and productivity as found by Sheridan and Bickford (2011). Hence, the general decrease observed in seedling density was likely due to the decline of forest specialists.

## 5. Conclusions

We found that the realised forest soil seed bank contained many generalist plants but also a considerable number of typical forest species. This evidence underpin the functional importance of the soil seed bank for forest understorey species, which has often been neglected. The proportion of forest specialists was generally higher in dense forests than open forests suggesting that forest management affects the germination responses from the seed bank and could help or prevent the

conservation of forest understorey diversity. Germination from the seed bank was influenced by edge microclimate in a contrasting way, depending on macroclimate. Generally, it shifted the seed bank seedling composition towards more light-demanding communities. Also, with simulated warming, a more light and temperature demanding community developed from the seed bank. These changes could alter, in the long term, the aboveground vegetation composition and dynamics, causing a thermophilization of the forest understorey and biodiversity loss. Remarkably, the seed bank of thermophilous forests was more plastic, due to the co-existence of numerous species that responded differently depending on how variation in environmental conditions affected their germination. We suggest that being more diverse, thermophilous forest seed banks could perhaps better cope with these environmental changes. Research on seed bank responses to edge effects in the face of climate warming can improve our ability to predict the impact of these changes and to design optimal forest management strategies. Further research on seed banks should consider forest edge and interior microclimates when quantifying the functional role of forest seed banks.

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## CRediT authorship contribution statement

**Cristina Gasperini:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft. **Elisa Carrari:** Methodology, Writing – review & editing. **Sanne Govaert:** Investigation, Writing – review & editing. **Camille Meeussen:** Investigation, Writing – review & editing. **Karen De Pauw:** Investigation, Writing – review & editing. **Jan Plue:** Conceptualization, Writing – review & editing. **Pieter Sanczuk:** Investigation, Writing – review & editing. **Thomas Vanneste:** Investigation, Writing – review & editing. **Pieter Vangansbeke:** Investigation, Writing – review & editing. **Giovanni Jacopetti:** Investigation, Writing – review & editing. **Pieter De Frenne:** Conceptualization, Investigation, Supervision, Writing – review & editing. **Federico Selvi:** Conceptualization, Investigation, Supervision, Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

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## References

- Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecol.* 26, 32–46. <https://doi.org/10.1111/j.1442-9993.2001.01070>. pp. x.
- Aussenac, G., 2000. Interactions between forest stands and microclimate: ecophysiological aspects and consequences for silviculture. *Ann. For. Sci.* 57, 287–301. <https://doi.org/10.1051/forest:2000119>.
- Barton, K., 2019. MuMIn: multi-model inference. R Packag. version 1.43.6. <http://cran.r-project.org/package=MuMIn> (accessed 08 February 2021).



- Euro Med, 2006. Euro Med PlantBase - the information resource for Euro-Mediterranean plant diversity. <http://www2.bgbm.org/EuroPlusMed/query.asp/> (accessed 03 February 2021).
- Meussen, C., Govaert, S., Vanneste, T., Calders, K., Bollmann, K., Brunet, J., Calders, K., Cousins, S.A.O., De Pauw, K., Diekmann, M., Gasperini, C., Graae, B.J., Hedwall, P.-O., Hylander, K., Iacopetti, G., Lenoir, J., Lindmo, S., Orczewska, A., Ponette, Q., Plue, J., Sanczuk, P., Selvi, F., Spicher, F., Verbeeck, H., Vermeir, P., Zellweger, F., Verheyen, K., Vangansbeke, P., De Frenne, P., 2021. Microclimatic gradients in European deciduous forest edges. *Agric. For. Meteorol.* (in press, Submitted).
- Mitlacher, K., Poschod, P., Rosén, E., Bakker, J.P., 2002. Restoration of wooded meadows - a comparative analysis along a chronosequence on Öland (Sweden). *Appl. Veg. Sci.* 5, 63–73. <https://doi.org/10.1111/j.1654-109X.2002.tb00536.x>.
- Naaf, T., Wulf, M., 2007. Effect of gap size, light and herbivory on the herb layer vegetation in European beech forest gaps. *For. Ecol. Manag.* 224, 141–149.
- Nakagawa, S., Schielzeth, H., 2013. A general and simple method for obtaining  $R^2$  from generalized linear mixed-effects models. *Methods Ecol. Evol.* 4, 133–142. <https://doi.org/10.1111/j.2041-210X.2012.00261.x>.
- Oksanen, J., Blanchet, F., Guillaume Friendly, M., Kindt, R., Legendre, P., McGlenn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solyomos, P., Henry, M., Stevens, H., Szoeck, E., Wagner, H., 2020. *Vegan: Community Ecology Package R Packag.*
- Olano, J.M., Caballero, I., Laskurain, N.A., Loidi, J., Escudero, A., 2002. Seed bank spatial pattern in a temperate secondary forest. *J. Veg. Sci.* 13, 775–784. <https://doi.org/10.1111/j.1654-1103.2002.tb02107.x>.
- Olden, J.D., Poff, N.L.R., Douglas, M.R., Douglas, M.E., Fausch, K.D., 2004. Ecological and evolutionary consequences of biotic homogenization. *Trends Ecol. Evol.* 19, 18–24. <https://doi.org/10.1016/j.tree.2003.09.010>.
- Ooi, M.K.J., 2012. Seed bank persistence and climate change. *Seed Sci. Res.* 22, S53–S60. <https://doi.org/10.1017/S0960258511000407>.
- Pakeman, R.J., Cummins, R.P., Miller, G.R., Roy, D.B., 1999. Potential climatic control of seedbank density. *Seed Sci. Res.* 9, 101–110. <https://doi.org/10.1017/S0960258599000112>.
- Panufnik-Medrzycka, D., Kwiatkowska-Falinska, A.J., 2014. The realised and potential soil seed bank in the potentilla albae-quercetum community in the Białowieża primeval Forest. *Acta Soc. Bot. Pol.* 70, 133–143. <https://doi.org/10.5586/asbp.2001.018>.
- Parker, V.T., Kelly, V.R., 1989. Seed banks in California Chaparral and other mediterranean climate shrublands. *Ecology of Soil Seed Banks*. Academic Press, Inc., pp. 231–255. <https://doi.org/10.1016/B978-0-12-440405-2.50016-6>.
- Parmesan, C., 2006. Ecological and evolutionary responses to recent climate change. *Annu. Rev. Ecol. Syst.* 37, 637–669. <https://doi.org/10.1146/annurev.ecolsys.37.091305.110100>.
- Pignatti, S., Menegoni, P., Pietrosanti, S., 2005. *Braun-Blanquetia. Bioindicator Values of Vascular Plants of the Flora of Italy*. Università degli studi di Camerino, Camerino.
- Pignatti, S., Guarino, R., La Rosa, M., 2017. *Flora d'Italia. 2nd edition*. Edagricole-New Business Media.
- Plue, J., Verheyen, K., Van Calster, H., Marage, D., Thompson, K., Kalamees, R., Jankowska-Blaszczuk, M., Bossuyt, B., Hermy, M., 2010. Seed banks of temperate deciduous forests during secondary succession. *J. Veg. Sci.* 21, 965–978. <https://doi.org/10.1111/j.1654-1103.2010.01203.x>.
- Plue, J., Thompson, K., Verheyen, K., Hermy, M., 2012. Seed banking in ancient forest species: why total sampled area really matters. *Seed Sci. Res.* 22, 123–133. <https://doi.org/10.1017/S0960258511000481>.
- Plue, J., Colas, F., Auffret, A.G., Cousins, S.A.O., 2017a. Methodological bias in the seed bank flora holds significant implications for understanding seed bank community functions. *Plant Biol.* 19, 201–210. <https://doi.org/10.1111/plb.12516>.
- Plue, J., De Frenne, P., Acharya, K., Brunet, J., Chabrierie, O., Decocq, G., Diekmann, M., Graae, B.J., Heinken, T., Hermy, M., Kolb, A., Lemke, I., Liira, J., Naaf, T., Verheyen, K., Wulf, M., Cousins, S.A.O., 2017b. Where does the community start, and where does it end? Including the seed bank to reassess forest herb layer responses to the environment. *J. Veg. Sci.* 28, 424–435. <https://doi.org/10.1111/jvs.12493>.
- Plue, J., Van Calster, H., Auestad, I., Basto, S., Bekker, R.M., Bruun, H.H., Chevalier, R., Decocq, G., Grandin, U., Hermy, M., Jacquemyn, H., Jakobsson, A., Jankowska-Blaszczuk, M., Kalamees, R., Koch, M.A., Marrs, R.H., Martensdóttir, B., Milberg, P., Måren, I.E., Pakeman, R.J., Phoenix, G.K., Thompson, K., Vandvik, V., Wagner, M., Auffret, A.G., 2021. Buffering effects of soil seed banks on plant community composition in response to land use and climate. *Glob. Ecol. Biogeogr.* 30, 128–139. <https://doi.org/10.1111/geb.13201>.
- Pykälä, J., 2004. Immediate increase in plant species richness after clear-cutting of boreal herb-rich forests. *Appl. Veg. Sci.* 7, 29–34. <https://doi.org/10.1111/j.1654-109X.2004.tb00592.x>.
- R Core Team, 2019. *R: A Language and Environment for Statistical Computing*. R Found. Stat. Comput. <https://www.R-project.org/>.
- Reinmann, A.B., Smith, I.A., Thompson, J.R., Hutyra, L.R., 2020. Urbanization and fragmentation mediate temperate forest carbon cycle response to climate. *Environ. Res. Lett.* 15, 114036. <https://doi.org/10.1088/1748-9326/abbf16>.
- Ritter, E., Dalsgaard, L., Einhorn, K.S., 2005. Light, temperature and soil moisture regimes following gap formation in a semi-natural beech-dominated forest in Denmark. *For. Ecol. Manag.* 206, 15–33. <https://doi.org/10.1016/j.foreco.2004.08.011>.
- Riutta, T., Slade, E.M., Bebbler, D.P., Taylor, M.E., Malhi, Y., Riordan, P., Macdonald, D.W., Morecroft, M.D., 2012. Experimental evidence for the interacting effects of forest edge, moisture and soil macrofauna on leaf litter decomposition. *Soil Biol. Biochem.* 49, 124–131. <https://doi.org/10.1016/j.soilbio.2012.02.028>.
- Rooney, T.P., Wiegmann, S.M., Rogers, D.A., Waller, D.M., 2004. Biotic impoverishment and homogenization in unfragmented forest understory communities. *Conserv. Biol.* 18, 787–798. <https://doi.org/10.1111/j.1523-1739.2004.00515.x>.
- Rustad, L.E., Campbell, J.L., Marion, G.M., Norby, R.J., Mitchell, M.J., Hartley, A.E., Cornelissen, J.H.C., Gurevitch, J., Alward, R., Beier, C., Burke, I., Canadell, J., Callaghan, T., Christensen, T.R., Fahnestock, J., Fernandez, I., Harte, J., Hollister, R., John, H., Ineson, P., Johnson, M.G., Jonasson, S., John, L., Linder, S., Lukewille, A., Masters, G., Melillo, J., Mickelsen, A., Neill, C., Olszyk, D.M., Press, M., Pregitzer, K., Robinson, C., Rygielwicz, P.T., Sala, O., Schmidt, I.K., Shaver, G., Thompson, K., Tingey, D.T., Verburg, P., Wall, D., Welker, J., Wright, R., 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia* 125, 543–562. <https://doi.org/10.1007/s004420000544>.
- Schmidt, M., Lischeid, G., Nendel, C., 2019. Microclimate and matter dynamics in transition zones of forest to arable land. *Agric. For. Meteorol.* 268, 1–10. <https://doi.org/10.1016/j.agrformet.2019.01.001>.
- Sheridan, J.A., Bickford, D., 2011. Shrinking body size as an ecological response to climate change. *Nat. Clim. Chang.* 1, 401–406. <https://doi.org/10.1038/nclimate1259>.
- Simpson, R.L., Leck, M.A., Parker, V.T., 1989. Seed banks: general concepts and methodological issues. *Ecology of Soil Seed Banks*, Elsevier, pp. 3–8. <https://doi.org/10.1016/B978-0-12-440405-2.50006-3>.
- Smith, I.A., Hutyra, L.R., Reinmann, A.B., Marrs, J.K., Thompson, J.R., 2018. Piecing together the fragments: elucidating edge effects on forest carbon dynamics. *Front. Ecol. Environ.* 16, 213–221. <https://doi.org/10.1002/fee.1793>.
- Ten Brink, D.-J., Hendriksma, H.P., Bruun, H.H., 2013. Habitat specialization through germination cueing: a comparative study of herbs from forests and open habitats. *Ann. Bot.* 111, 283–292. <https://doi.org/10.1093/aob/mcs253>.
- Torras, O., Gil-Tena, A., Saura, S., 2008. How does forest landscape structure explain tree species richness in a Mediterranean context? *Biodivers. Conserv.* 17, 1227–1240. <https://doi.org/10.1007/s10531-007-9277-0>.
- Vandelook, F., Van de Moer, D., Van Assche, J.A., 2008. Environmental signals for seed germination reflect habitat adaptations in four temperate Caryophyllaceae. *Funct. Ecol.* 22, 470–478. <https://doi.org/10.1111/j.1365-2435.2008.01385.x>.
- Vandvik, V., Klanderud, K., Meineri, E., Måren, I.E., Tøpper, J., 2016. Seed banks are biodiversity reservoirs: species-area relationships above versus below ground. *Oikos* 125, 218–228. <https://doi.org/10.1111/oik.02022>.
- Walck, J.L., Hidayati, S.N., Dixon, K.W., Thompson, K., Poschod, P., 2011. Climate change and plant regeneration from seed. *Glob. Chang. Biol.* 17, 2145–2161. <https://doi.org/10.1111/j.1365-2486.2010.02368.x>.
- Yan, Q., Zhu, J., Gang, Q., 2013. Comparison of spatial patterns of soil seed banks between larch plantations and adjacent secondary forests in Northeast China: implication for spatial distribution of larch plantations. *Trees - Struct. Funct.* 27, 1747–1754. <https://doi.org/10.1007/s00468-013-0920-y>.
- Zellweger, F., de Frenne, P., Lenoir, J., Vangansbeke, P., Verheyen, K., Bernhardt-Römermann, M., Baeten, L., Hédli, R., Berki, I., Brunet, J., van Calster, H., Chudomelová, M., Decocq, G., Dirnböck, T., Durak, T., Heinken, T., Jaroszewicz, B., Kopecký, M., Málíš, F., Macek, M., Malicki, M., Naaf, T., Nagel, T.A., Ortmann-Ajkai, A., Petřík, P., Pielech, R., Raczynska, K., Schmidt, W., Standovář, T., Swierkosz, K., Teleki, B., Vild, O., Wulf, M., Coomes, D., 2020. Forest microclimate dynamics drive plant responses to warming. *Science (80-)* 368, 772–775. <https://doi.org/10.1126/science.aba6880>.
- Zou, C., Martini, F., Xia, S.W., Castillo-Diaz, D., Goodale, U.M., 2021. Elevation and micro environmental conditions directly and indirectly influence forests' soil seed bank communities. *Glob. Ecol. Conserv.* 26. <https://doi.org/10.1016/j.gecco.2020.e01443>.
- Zuur, A.F., Ieno, E.N., Elphick, C.S., 2010. A protocol for data exploration to avoid common statistical problems. *Methods Ecol. Evol.* 1, 3–14. <https://doi.org/10.1111/j.2041-210X.2009.00001.x>.