

Low recruitment across life stages partly accounts for the slow colonization of forest herbs

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Summary

1. Former land use has long-lasting effects on the distribution and abundance of forest herbs. Previous studies mainly focussed on limited dispersal capacities of forest herbs to explain these patterns and few studies have experimentally evaluated the relative importance of recruitment. Introduction experiments offer a direct test of recruitment limitation, but are generally only monitored until the germination stage.

2. We examined recruitment of 10 forest herbs during five growing seasons by means of a seed sowing experiment in two contrasting forest types (valley and plateau) established on former agricultural land. Effects of seed density and clearing of vegetation and litter (disturbed microsites) were tested in a factorial design. The data were analysed in two successive steps: germination 2 years after seed introduction and subsequent recruitment into the adult life stage. We focussed on both the species and the community-level.

3. Although adding more seeds resulted in more recruits, only a small fraction of seeds (between 1% and 20%) germinated after two growing seasons in both forest types. The need for disturbed microsites was species-specific and differed between the two forest types. Evergreen hemicryptophytes benefited from clearing; vernal geophytes were not affected. Post-seedling mortality further reduced recruitment success into the adult life stage. Results at the species and community-level were largely analogous.

4. Because only a fraction of seeds effectively recruited into the adult life stage, forest herbs need high seed densities for colonization. Seed availability is, however, severely limited by the low dispersal capacity of many forest herbs. Disturbed microsites promoted recruitment of some, but not all forest herbs. The effect was most clear in valley forest, probably because the established vegetation had high cover and was dominated by competitive herbs.

5. *Synthesis.* Overall, the experimental results indicate that community assembly can be considered a two-stage process in which restricted seed availability followed by low recruitment limits colonization. The need for disturbed microsites can be crucial, but depends on species-specific life histories and the prevailing site conditions.

Key-words: central Belgium, competition, dispersal limitation, forest herb colonization, per-seed-response, secondary forest succession, seed sowing experiment

Introduction

It is well known that human disturbances have long-lasting effects on community composition and structure of temperate deciduous forests (e.g. Brewer 1980; Duffy & Meier 1992;

Foster *et al.* 1998; Dupouey *et al.* 2002; Hall *et al.* 2002). Besides the obvious consequences for conservation, sites recovering from disturbance provide ideal settings for assessing key-drivers of community assembly because species need to recolonize unoccupied habitat patches. More generally, it is questioned to which extent species distributions and abundances are explained by dispersal or by environmentally

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controlled recruitment. Phases of forest clearance for agriculture followed by abandonment and afforestation formed the most severe and extensive human disturbance across European and eastern North American landscapes during the past centuries (e.g. Whitney 1994; Kirby & Watkins 1998). Numerous studies on both continents found that forest herb diversity in these post-agricultural forests remains impoverished for decades to centuries because many forest herbs have a low colonization capacity (reviewed in Flinn & Vellend 2005; Hermy & Verheyen 2007). A negative relationship between species occurrence and spatio-temporal isolation from a colonization source (e.g. Peterken & Game 1984; Matlack 1994; Brunet & Von Oheimb 1998; Bossuyt *et al.* 1999; Verheyen & Hermy 2001a,b) has led to the view that dispersal limitation principally underlies this low colonization capacity. More recently, however, evidence is growing that low recruitment success can additionally control the colonization process. Flinn (2007), for instance, showed that recruitment rates of three fern species differed as predicted by colonization capacities, that is, the slowest fern species had the lowest recruitment. Similarly, in an 8-year introduction experiment Baeten *et al.* (in press) related the contrasting colonization capacity of two forest herbs to differences in recruitment success.

Obviously, both dispersal limitation and recruitment simultaneously affect species distributions and abundances in post-agricultural forests and are not mutually exclusive explanations for community assembly (Eriksson & Ehrlén 1992; Clark *et al.* 2007). The point at issue is thus their relative importance. Testing the relative importance of both processes within the context of recovering forest plant communities can be done through observational studies (e.g. Verheyen & Hermy 2001a,b; Verheyen *et al.* 2003b) and more directly through seed sowing experiments (Donohue *et al.* 2000; Graae *et al.* 2004; Verheyen & Hermy 2004). As these experiments found successful seedling emergence at post-agricultural sites, it is clear that seed availability is critical to forest plant colonization.

The extent of recruitment limitation is, however, not explicitly covered by this result and some key-points need more emphasis. First, it is unlikely that all seeds reach the seedling stage, which implies that there are post-dispersal processes limiting recruitment. In a meta-analysis of seed sowing experiments across vegetation types, Clark *et al.* (2007) showed that generally only a small fraction of seeds effectively recruits into the seedling stage. This suggests that recruitment also plays a major role. Second, the suitability of a patch for recruitment might change between the seed, seedling and adult life stages, which is conceptually termed 'life stage conflicts' (Schupp 1995) or 'ontogenetic niche shifts' (Young *et al.* 2005). Successful recruitment into the seedling stage does therefore not exclude recruitment to be hampered in subsequent phases (Graae *et al.* 2004; Baeten *et al.* in press). Third, the extent of recruitment limitation of many forest herbs has proven to be dependent on the availability of temporary disturbed microsites (e.g. Eriksson & Ehrlén 1992; Verheyen & Hermy 2004). The importance of these microsites might, however, be different between contrasting forest types. The effect of removing competing vegetation can vary, for

instance, between forests differing in total herb layer cover or dominant species. In addition, it is unknown whether or not microsites following small-scale disturbance need to be persistent to allow further recruitment into the adult stage.

The central subject of this paper is the evaluation of recruitment limitation of forest herbs according to these processes. We present results of a seed sowing experiment including ten forest herbs introduced in two contrasting post-agricultural forest types: valley and plateau forest. Seeds were sown in three species-specific densities and plots were cleared from litter and vegetation to create disturbed microsites or were left undisturbed. Initial recruitment success 2 years after seed sowing was analysed at the species and experimental community-level. Then, we evaluated further recruitment into the adult life stage following five growing seasons. We hypothesized that: (i) only a minor fraction of seeds can successfully recruit; (ii) adding more seeds and creating disturbed microsites results in higher recruitment; (iii) recruitment of adult individuals after five growing seasons is lower than expected from initial germination results.

Methods

STUDY SITE AND SPECIES

The seed sowing experiment was carried out in a forested landscape 20 km east of Brussels (central Belgium), situated on a loamy belt that extends over the central part of Belgium. Two contrasting forest types were included in this study: (i) 'valley' forest on rich, moist soils and (ii) 'plateau' forest on less rich, drier soils. Each forest type was represented by two recent forest stands within the study region, that is, stands that were established on former agricultural land after 1850 (plateau) or 1909–1940 (valley).

The two valley forest stands were located in the 'Doode Bemde', a nature reserve situated along the lowland river Dijle. The relation between the present day distribution of forest herbs within this area and historical land-use are discussed in Verheyen *et al.* (2003b). Main canopy species in the study sites were *Populus* spp. and *Alnus glutinosa* (L.) Gaertn, which formed a thin litter layer (type: mésomull, Jabiol *et al.* 1995). Average nutrient concentrations (\pm SE, mg/100 g dry soil) based on eight mixed 0–10 cm soil samples were 389.0 ± 20.0 total N (Kjeldahl method) and 15.4 ± 1.8 K⁺, 23.0 ± 2.1 Mg²⁺ and 271.2 ± 40.4 Ca²⁺ (ammonium-acetate EDTA extraction, Van Ranst *et al.* 1999). Average phosphorus concentration (ammonium-acetate EDTA) was 2.1 ± 0.3 mg/100 g dry soil, which is three times higher than the phosphorus levels in forest without agricultural history (c. 0.75 mg P/100 g dry soil, Baeten *et al.* in press). Site conditions were characteristic for a mesotrophic, moist, alluvial forest type (*Alno-Padion sensu* Noirfalise 1984).

The two drier forest stands were located on the surrounding plateau, one of which was adjacent to the Meerdaal forest. Bossuyt *et al.* (1999) present detailed results on herb species colonization patterns within this area. The canopy species was *Fagus sylvatica* L., which formed a thick litter layer (type: dysmoder, Jabiol *et al.* 1995). Average nutrient concentrations were 7.1 ± 1.9 P, 171.7 ± 25.7 total N, 10.0 ± 1.1 K⁺, 3.0 ± 0.6 Mg²⁺ and 21.7 ± 3.6 Ca²⁺ (\pm SE, mg/100 g dry soil). Site conditions were characteristic for upland, relatively dry and acidic forests on deep loamy soils naturally dominated by beech (*Milio-Fagetum sensu* Noirfalise 1984).

Table 1. Forest herbs that are included in this study. For each species, the forest type in which it was sown, seed sowing densities (seeds m⁻²), life-form and leaf phenology are indicated

Species	Forest type	Seed densities		Life-form	Leaf phenology
<i>Anemone nemorosa</i>	Valley and Plateau	200, 1000, 4000	Herb	Geophyte	Vernal
<i>Hyacinthoides non-scripta</i>	Valley and Plateau	80, 400, 1600	Herb	Geophyte	Vernal
<i>Carex sylvatica</i>	Valley	200, 1000, 4000	Graminoïd	Hemicytrophite	Evergreen
<i>Geum urbanum</i>	Valley	200, 1000, 4000	Herb	Hemicytrophite	Evergreen
<i>Paris quadrifolia</i>	Valley	36, 180, 720	Herb	Geophyte	Aestival
<i>Primula elatior</i>	Valley	200, 800, 1600	Herb	Hemicytrophite	Evergreen
<i>Lamium galeobdolon</i>	Plateau	60, 300, 1200	Herb	Chamaephyte	Evergreen
<i>Milium effusum</i>	Plateau	200, 1000, 4000	Graminoïd	Hemicytrophite	Evergreen
<i>Oxalis acetosella</i>	Plateau	40, 200, 800	Herb	Chamaephyte	Evergreen
<i>Polygonatum multiflorum</i>	Plateau	28, 140, 560	Herb	Geophyte	Aestival

Differences in productivity between valley and plateau forest were reflected in cover and composition of the herb layer. During a vegetation survey conducted in May 2007, each species was recorded together with its estimated percentage cover in each experimental plot. Average herb layer cover in valley forest was 80% ($\pm 2\%$ SE) and in plateau forest only 34% ($\pm 4\%$ SE). A second critical difference between both forest types was the dominance of the competitive herb *Urtica dioica* L. (cf. De Keersmaecker *et al.* 2004), which covered on average 35% ($\pm 4\%$ SE) in valley forest, whereas this herb was absent from plateau forest.

Ten characteristic forest herbs, six in each forest type, were introduced as seeds. Two species, *Hyacinthoides non-scripta* L. and *Anemone nemorosa* L., were sown in both forest types. Details on species-specific seed sowing densities, growth form and leaf phenology are given in Table 1. All, except for *Geum urbanum* L., have proven to be slow colonizing forest herbs (Verheyen *et al.* 2003c), which are generally confined to ancient forest sites. Species with different life-forms (geophytes, hemicytrophites and chamaephytes) and leaf phenology were selected to exclude bias in our results towards certain species groups.

DESIGN AND MONITORING

The experiment was initiated during 2002. The design corresponds to a factorial experiment with the treatments fully crossed: (i) the vegetation and litter was cleared or left intact ('cleared' and 'undisturbed') and (ii) seeds were sown in three species-specific densities (Table 1). In half of the experimental 1.75 m \times 3.25 m plots, all vegetation and litter were removed to create disturbed microsites of bare ground. This was done in both valley and plateau forest. Seeds were collected at ancient forest sites near the experimental sites and were sown immediately. Collection occurred between spring and summer 2002 after seed ripening; this is also the period in which seeds are naturally dispersed. The resulting six clearing \times seed density treatment combinations were replicated eight times for each forest type. These 48 plots per forest type were equally distributed over the two forest stands of each forest type. For practical reasons, plots were aggregated into two groups of 12 plots in every forest stand and fenced with a 1.5-m high enclosure. Within every plot, the six different forest herbs were sown in separate 0.5 m \times 0.5 m subplots. All plots and subplots were permanently marked.

In each subplot, the total number of individuals was counted yearly (2003–2007) in spring. For *Oxalis acetosella* L., *Lamium galeobdolon* (L.) L. and *Carex sylvatica* Huds. counts were only

performed in 2003–2004 because afterwards, strong vegetative growth and a clumped distribution made the distinction of separate individuals unreliable. *Paris quadrifolia* L. showed very limited recruitment success, only 12% of the plots contained recruits in 2004, so the species was not further monitored. Individuals of *Primula elatior* (L.) Hill and *G. urbanum* were recorded in three different life stages (seedlings, non-reproductive and reproductive individuals) to obtain more detailed demographic data. These species were specifically chosen because they show limited vegetative growth, form rosettes that can easily be distinguished and showed relatively high recruitment success.

DATA ANALYSIS

The data were analysed in two successive steps: (i) germination success 2 years after seed sowing and (ii) ultimate recruitment of adult individuals after five growing seasons. Furthermore, the analysis of both steps was performed at the subplot (individual species) and the plot (experimental community) level. 'Forest type' was not included as a factor throughout the analysis because the species' natural occurrences are mostly confounded with forest type – only two species could be sown in both valley and plateau forest – and because 'forest type' had only two true observations (stands).

The effects of clearing and seed density on the number of recruits after 2 years (2004) in each plot were tested using permutational analysis of variance (ANOVA) (Anderson 2001; McArdle & Anderson 2001). The use of this technique instead of traditional ANOVA was preferred because the data contained many zeros, the distribution of observations was highly skewed and counts take discrete values rather than being continuous. The additive partitioning of total variance in the dataset is analogous to ANOVA. The appropriate sum of squares is calculated from a matrix containing distances between samples (here subplots) based on the measured variables (number of recruits). The significance of the resulting test statistic is tested through permutation (999 randomizations). We used the Euclidean distance measure for calculating the distance matrix, resulting in the same test statistic as the traditional parametric univariate *F*-ratio (Anderson 2001). Tests were performed with the FORTRAN-based program PERMANOVA (Anderson 2001; McArdle & Anderson 2001).

We calculated the per-seed-response measure proposed by Clark *et al.* (2007) to evaluate whether initial germination overestimated recruitment success. Hereto, differences in per-seed-response 2 years (2004) and 5 years (2007) after seed sowing were tested with a non-parametric Wilcoxon Signed-Rank test in spss 15.0 statistical software.

Table 2. Effect of vegetation and litter clearing and seed density on the number of recruits of each species 2 years after seed sowing. Test statistic: pseudo-*F* value based on a permutational analysis of variance (Anderson 2001); significance tested through 999 randomizations (NS, not-significant; * $P \leq 0.05$; ** $0.001 < P \leq 0.01$; *** $P \leq 0.001$). A positive effect means higher numbers of recruits in cleared plots or at higher seeds densities.

Species	Clearing		Seed density		Clearing × seed density
	Effect	<i>F</i>	Effect	<i>F</i>	<i>F</i>
(a) Valley					
<i>Anemone nemorosa</i>		0.55 ^{NS}		1.12 ^{NS}	2.64 ^{NS}
<i>Hyacinthoides non-scripta</i>		0.38 ^{NS}	+	4.54*	0.02 ^{NS}
<i>Carex sylvatica</i>	+	6.54***	+	3.15**	3.24**
<i>Geum urbanum</i>	+	23.96***	+	5.79**	3.01 ^{NS}
<i>Primula elatior</i>	+	13.32***	+	3.67*	1.97 ^{NS}
(b) Plateau					
<i>Anemone nemorosa</i>		0.55 ^{NS}		0.39 ^{NS}	0.04 ^{NS}
<i>Hyacinthoides non-scripta</i>		0.08 ^{NS}	+	48.45***	1.17 ^{NS}
<i>Polygonatum multiflorum</i>	+	6.27*	+	9.91***	0.41 ^{NS}
<i>Oxalis acetosella</i>		2.28 ^{NS}		1.38 ^{NS}	1.77 ^{NS}
<i>Lamium galeobdolon</i>		1.18 ^{NS}	+	4.00*	2.20 ^{NS}
<i>Milium effusum</i>		0.24 ^{NS}	+	4.77*	0.55 ^{NS}

The per-seed-response measure is calculated as the difference between the number of recruits in experimental plots and control plots standardised by the number of seeds added. Since in our study sites the sown species were absent from control plots, this measure simplifies to the number of recruits per seed. A per-seed-response measure was specifically chosen because it has proven to work best if seed augmentation levels are relatively low (cf. maximum 4000 seeds m⁻² in this study) so that seed addition does not lead to complete saturation (Clark *et al.* 2007). In 2004, all individuals originated from the experimentally added seeds whereas in 2007 individuals could already have originated from reproductive adults. Therefore, the per-seed-response for 2007 was calculated with the number of adults, which most likely originated from the sown seeds, instead of with the total number of individuals. Furthermore, we assessed density-dependence of mortality between the seedling and adult life stage. Hereto, one minus the proportion of adults in 2007 to the number of recruits in 2004, that is, post-seedling mortality, was related to initial seed density by means of bivariate Spearman Rank correlations in SPSS 15.0 statistical software.

The count data of subplots, containing the six different forest herbs, were combined for every plot. This plot level can be viewed as an experimental community in which forest herbs are sown in spatially separated locations. Differences between plots based on (sown) species composition were visualized with PCA in CANOCO 4.5 ordination software. This technique was preferred because it gives a good visualization of intercorrelations between species when species scores are divided by standard deviation (Lepš & Šmilauer 2003). The assumption of linearity of the data was met. Effects of clearing and seed density on plot scores along the PCA axes were tested with nonparametric Kruskal–Wallis tests in SPSS 15.0 statistical software. To test for effects of clearing and seed density on overall recruitment at the plot level we ran a permutational MANOVA (Anderson 2001). Plots were included as samples, the number of individuals of each species as separate variables. Multivariate distances between plots were again calculated using the Euclidean measure and significances were tested through permutation (999 randomizations). Tests were

performed with the FORTRAN-based program PERMANOVA (Anderson 2001; McArdle & Anderson 2001).

Results

GERMINATION AT THE SPECIES AND COMMUNITY-LEVEL

In the moist valley forest type the sown herbs, except for the two vernal geophytes *H. non-scripta* and *A. nemorosa*, showed significantly higher germination in cleared plots (Table 2). Apart from *A. nemorosa*, higher seed densities resulted in more recruits. The significant interaction for *C. sylvatica* resulted from a stronger seed density effect in cleared compared to undisturbed plots. In the dry plateau forest type, vegetation and litter clearing did only affect germination success of *Polygonatum multiflorum* (L.) All., whereas higher seed densities resulted in more recruits in four out of six species.

Results at the level of the experimental community, combining all species at the plot level, were largely analogous (Table 3a). When testing the number of recruits of all species simultaneously with permutational MANOVA, it appeared that in valley forest both clearing and seed density affected composition at the plot level. Cleared and undisturbed plots were significantly separated along the second PCA axis (Fig. 1; $\chi^2 = 23.27$, $P < 0.001$) whereas seed density did not significantly separate along the first and second axis. Figure 1 is a correlation bi-plot representing species–species and species–plot correlations. In the plateau forest type, only seed density had a (highly) significant effect on the number of recruits per plot across species (Table 3a). Species abundances were all positively intercorrelated (Fig. 1) and seed densities separated significantly along the first PCA axis ($\chi^2 = 31.90$, $P < 0.001$).

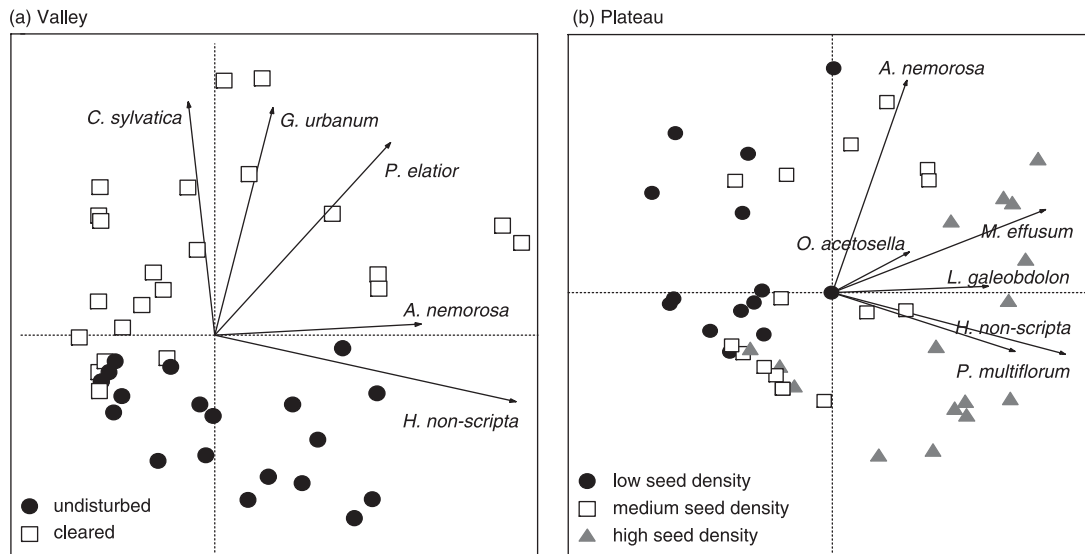


Fig. 1. Correlation bi-plot of a PCA analysis on the number of recruits of the sown species in each plot 2 years after seed sowing. The first two ordination axes account for 69.3% (a) and 70.1% (b) of the total variance. See Table 2 for full species names

Table 3. Effects of vegetation and litter clearing and seed density on the number of individuals of all species combined at the plot level tested with permutational MANOVA. Test statistic: pseudo- F value based on a permutational analysis of variance (Anderson 2001); significance tested through 999 randomizations (NS, not significant; * $P \leq 0.05$; *** $P \leq 0.001$).

Source of variation	Valley forest	Plateau forest
(a) Germination (2004 data)		
Clearing	3.68*	0.47 ^{NS}
Seed density	4.04***	42.11***
Clearing \times seed density	1.11 ^{NS}	1.08 ^{NS}
(b) Recruitment into the adult life stage (2007 data)		
Clearing	0.81 ^{NS}	0.23 ^{NS}
Seed density	3.90***	12.77***
Clearing \times seed density	0.16 ^{NS}	0.06 ^{NS}

RECRUITMENT TO THE ADULT LIFE STAGE: CHANGE 2004–2007

In cleared plots, the per-seed-response of the two hemicryptophytes *G. urbanum* and *P. elatior* significantly decreased between 2004 and 2007 (Fig. 2). Thus, for these forest herbs the number of recruits per seed 2 years after seed sowing was higher than the number of recruits per seed that remained after 5 years. In undisturbed plots, the initial low per-seed-response of the hemicryptophytes did not further decrease. The per-seed-response of the two vernal geophytes *H. non-scripta* and *A. nemorosa*, which were sown in both the valley and plateau forest type, significantly decreased in the dry plateau forest type only. Furthermore, in the plateau forest type, per-seed-response did change significantly between 2004 and 2007 for all species except *Milium effusum* L. Only for *H. non-scripta* in the plateau forest type, the proportion of recruits in 2004

that did not grow into an adult life stage in 2007 was significantly related to initial seed density. In cleared plots and undisturbed plots the Spearman correlation coefficients were -0.642 ($P < 0.001$) and -0.540 ($P = 0.008$), respectively. Thus, post-seedling mortality, that is, the proportion of individuals that did not survive to the adult stage (2007), was lower at higher seed densities.

In the valley forest type, recruitment success of the hemicryptophytes in cleared plots decreased between 2004 and 2007; clearing did no longer significantly affect composition at the plot level (Table 3b). Seed density remained a significant source of variation on the overall number of individuals in both valley and plateau forest; a higher seed density resulted in more recruits.

In undisturbed plots, the number of individuals across the three life stages of *G. urbanum* and *P. elatior* was low (Fig. 3). Only a low number of seedlings and vegetative individuals recruited 2 years after seed sowing (2004) so that further growth to a reproductive stage (2007) was very limited. In cleared plots, recruitment 2 years after seed sowing (2004) of both *G. urbanum* and *P. elatior* was more successful (cf. Table 2). However, the distribution of both species across the three life stages differed between 2004 and 2007. Seedlings of *G. urbanum* were found in every year up to 2007, but seedlings of *P. elatior* were mostly missing from 2005 onwards.

Discussion

The experimental results presented in this study clearly indicate that dispersal constraints can severely limit colonization of forest herbs. Higher seed densities resulted in more recruits in eight out of 10 species and positively affected abundances at the plot level. The positive effect of seed availability on species abundances persisted at least for 5 years following seed sowing. Our results thus agree with the extensive work on

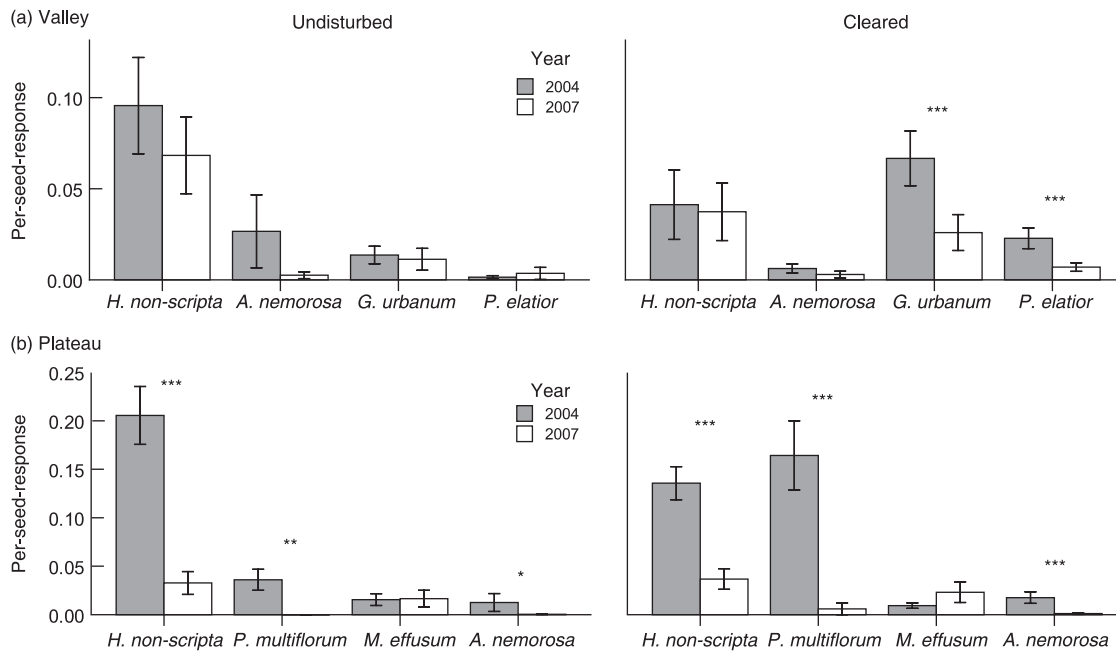


Fig. 2. Average number of individuals per subplot in 2004 and 2007 standardized by the number of seeds added (per-seed-response). Significant differences between years (Wilcoxon Signed-Rank) are given above bars; * $P \leq 0.05$; ** $0.001 < P \leq 0.01$; *** $P \leq 0.001$. Error bars represent standard errors. See Table 2 for full species names

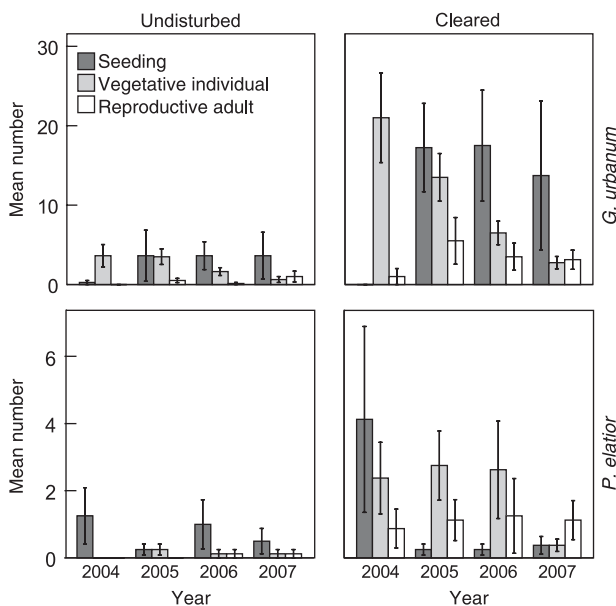


Fig. 3. Distribution of all individuals per subplot of *Geum urbanum* (4000 seeds m^{-2}) and *Primula elatior* (1600 seeds m^{-2}) across tree life stages between 2004 and 2007. Error bars represent standard errors.

recovering forests confirming the major importance of dispersal limitation to explain distributional patterns of forest herbs (e.g. Matlack 1994; Bossuyt *et al.* 1999; Verheyen & Hermy 2001a,b). A key point to be made, however, is that our experimental results strongly suggest that dispersal limitation alone offers a too conservative explanation for the low colonization capacity of forest herbs. The low per-seed-responses, limited recruitment into an adult life stage, beneficial

effects of vegetation and litter clearing, and ephemeral recruitment success for *P. elatior* underpin this suggestion.

GERMINATION SUCCESS

If seed sowing in previously unoccupied sites results in successful seedling emergence, one can conclude that the site is actually suitable for recruitment and the absence of the sown species can be attributed to seed limitation (Ehrlén & Eriksson 2000). This observation, however, does not provide information on the relative importance of seed limitation versus other factors limiting recruitment. In this respect, a per-seed-response is more informative (Clark *et al.* 2007). In the two studied forest types, the proportion of seeds that could successfully recruit did not exceed 20% and was more often lower than 5%. In a similar seed sowing experiment including three of our study species, Verheyen & Hermy (2004) also found that the number of seeds developing into seedlings ranged between 10% and 20%. Donohue *et al.* (2000) reported higher recruitment success (32%) of *Gaultheria procumbens* L., but no seedlings survived the first growing season. Our seed densities were somewhat higher compared to similar seed sowing experiments (Graae *et al.* 2004, 40–100 seeds m^{-2} ; Verheyen & Hermy 2004, 144–400 seeds m^{-2}), but are still realistic for natural populations. Across the studied species, on average 10 ± 4 SE reproductive individuals or ramets would be sufficient to produce seed numbers of the intermediate seed density treatment (average seed production per plant from Fitter & Peat 1994). Nevertheless, we lack exact seed production data and recommend for future studies to couple seed sowing experiments with the quantification of the ambient seed rain. Based on the low recruitment success,

it is clear that the stage between seed arrival and the recruitment of seedlings is critical for recovering forest plant populations (Harper 1977). Seed dispersal is a primary condition to enable colonization – seeds must arrive at a site to germinate – but other post-dispersal factors and processes such as seed viability, pathogens, predation and microsite availability obviously shape a second stage bottle-neck for forest recovery (cf. Verheyen *et al.* 2003b; Baeten *et al.* in press).

The need for disturbed microsites for recruitment was tested by clearing litter and vegetation in half of the plots. Our data for valley forests confirm the findings of Verheyen & Hermy (2004) on the difference in the effect of clearing on the germination of evergreen hemicryptophytes and vernal geophytes. Germination of the three hemicryptophytes was positively affected by clearing, which supports the idea that these species need a spatial regeneration niche for successful recruitment. Several mechanisms might be involved in the response of species to the clearing treatment. Some lines of evidence suggest, however, that reduced competition at least partially plays an important role. First, vernal species avoid competition through phenological escape (Pigott 1982) and were, correspondingly, not affected by the biomass removal in our experiment. This difference in strategy probably accounts for the contrasting intercorrelation with the other species and the independence of the clearing treatment (Fig. 1a). Second, a vegetation survey in all plots showed that the competitive herb *U. dioica* dominated our experimental plots (average cover $35\% \pm 4\%$ SE). This species is known to profit from high phosphorus availability, a persistent legacy from former agricultural land-use, and can hamper the colonization of forest herbs (e.g. Hermy *et al.* 1999; Honnay *et al.* 1999; De Keersmaecker *et al.* 2004; Baeten *et al.* in press). Phosphorus is therefore an indirect, via *U. dioica*, hindrance to community recovery. Slow colonization of forest herbs following other forms of past land use such as clearcutting (e.g. Duffy & Meier 1992) will be related to other (indirect) effects like reduced small-scale environmental heterogeneity (e.g. Flinn 2007; Flinn & Marks 2007) or interactions with mycorrhizae, herbivores, pollinators and pathogens (Flinn & Vellend 2005). Many of these factors remain, however, poorly studied.

In plateau forest, clearing only affected germination of one out of six species. Clearing included the removal of the litter layer, which was reasonably thick in plateau forest, and created plots with bare soil. This is an important difference with valley forest in which the litter layer was nearly absent. Because the sown species in plateau forest have proven to grow well at sites with large litter accumulation (Packham 1978; Sydes & Grime 1981), the absence of beneficial effects of litter removal are not surprising. Verheyen *et al.* (2003a) suggested that slow recovery in unproductive oak forest was partially the result of the thick litter layer preventing recruitment. Our experimental data do not support this idea. Reducing competition through vegetation removal was probably less important in unproductive plateau forest because total herb layer cover was low and *U. dioica* was absent. Fraterrigo *et al.* (2006), for instance, studied allocation in forest herbs and also found no evidence of competition for light between forest

herbs in recovering forest with sparse herb layer cover. As a result, only seed density affected germination success in plateau forest, resulting in high intercorrelations between species abundances at the plot level (Fig. 1b). Differences in the effect of clearing between forest types thus presumably stem from differences in the type of biomass that is mainly removed (litter or vegetation) and the species-specific ecological characteristics.

RECRUITMENT TO THE ADULT LIFE STAGE

The number of adults that recruited 5 years after seed sowing was generally lower than the number of recruits observed after 2 years. As suggested by Clark *et al.* (2007), post-seedling mortality indeed further decreased the per-seed-response and thus the importance of seed limitation. This mortality could be partly the result of life stage conflicts: patches that are suitable for recruitment into one life stage might be unsuitable for a subsequent stage (Schupp 1995). To unravel which life stage transitions formed a bottle-neck for recruitment, however, more detailed demographic analyses with higher frequency of sampling would be required. Post-seedling mortality did not result from density-dependence (Crawley 2003) as the proportion of recruits that could not grow into the adult life stage did not increase with seed density. In fact, recruitment success to the adult stage of *H. non-scripta* in plateau forest increased with seed density indicating positive density-dependence. The strong decrease in per-seed-response of this herb between 2004 and 2007 (Fig. 2) could therefore not be attributed to density-dependence. Although seed densities were reasonably high, subplots were apparently not yet saturated. Even though the nature of post-seedling mortality remains largely unstudied in this experiment, it is obvious that massive amounts of seeds must reach recovering forests to generate only a few adult individuals. Seed availability is, however, restricted by the limited capacity of many forest herbs to disperse (e.g. Verheyen *et al.* 2003c) so that a low per-seed-response imposes important constraints for recovery. This especially applies to highly fragmented landscapes (e.g. Honnay *et al.* 2002).

It has been suggested in other seed sowing experiments that certain forest herbs need disturbed microsites or spatial regeneration niches to germinate (Eriksson & Ehrlén 1992; Verheyen & Hermy 2004). As discussed earlier, our data from valley forest support this view, but add to this that persistence of such microsites is probably needed for further recruitment. Species that initially benefitted from vegetation and litter removal showed a significant decrease in per-seed-response during further recruitment into an adult life stage (Fig. 2). Therefore, only 5 years after microsite creation, abundances of the sown species did no longer differ between cleared and undisturbed plots (Table 3b). In the plateau forest type, only one species initially benefitted from clearing. Per-seed-response of this species, however, also drastically decreased in cleared plots so that the per-seed-response of cleared and undisturbed plots became similar.

The number of adults that could recruit after 5 years does not completely reflect population persistence in the longer

term. The distributions of *G. urbanum* and *P. elatior* across life stages are more informative in this context (Fig. 3). Both species mainly recruited in cleared plots, but a different distribution across life stages reflects their contrasting recruitment success. For *G. urbanum*, new seedlings were found until 2007 indicating that this species could successfully reproduce. Recruitment success of *P. elatior*, on the other hand, seemed rather ephemeral. Some reproductive adults persisted, but seedlings became scarce. Persistence of this species is therefore uncertain if future mortality of adults is not compensated for by recruitment. These findings fully underpin experimental data from Baeten *et al.* (in press) showing that *G. urbanum* can counterbalance lower survival in post-agricultural forest by new recruitment, whereas *P. elatior* fails to do so. Therefore, it was concluded that this difference in recruitment success contributes to the differential colonization capacity of *G. urbanum* and *P. elatior*, which are fast and slow colonizing species, respectively.

In conclusion, we showed that dispersal limitation alone is insufficient to account for the low colonization rates of forest herbs during forest recovery. Rather, limited dispersal coupled with a small fraction of seeds that effectively recruits into the adult life stage explained establishment. Our experimental data thus provide a useful test of the synthetic framework proposed by Cramer *et al.* (2008) in which thresholds of dispersal and recruitment interacting with legacies from former land use are combined to explain community assembly following the abandonment of land. Therefore, we argue that post-agricultural forests form particular study systems that are similar to a great number of other systems in which species do not recover after human disturbances and abandonment. Furthermore, we found that post-seedling mortality significantly contributed to the low recruitment success. This result stresses the importance of long-term monitoring and indicates that previous studies, focusing on the germination stage, might have underestimated the relative importance of recruitment. Assessing recruitment into an adult life stage is, however, insufficient to fully evaluate population viability in post-agricultural forests. We therefore agree with Flinn & Vellend (2005) on the importance of demographic studies determining rates between different life stages as an important direction for future research.

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