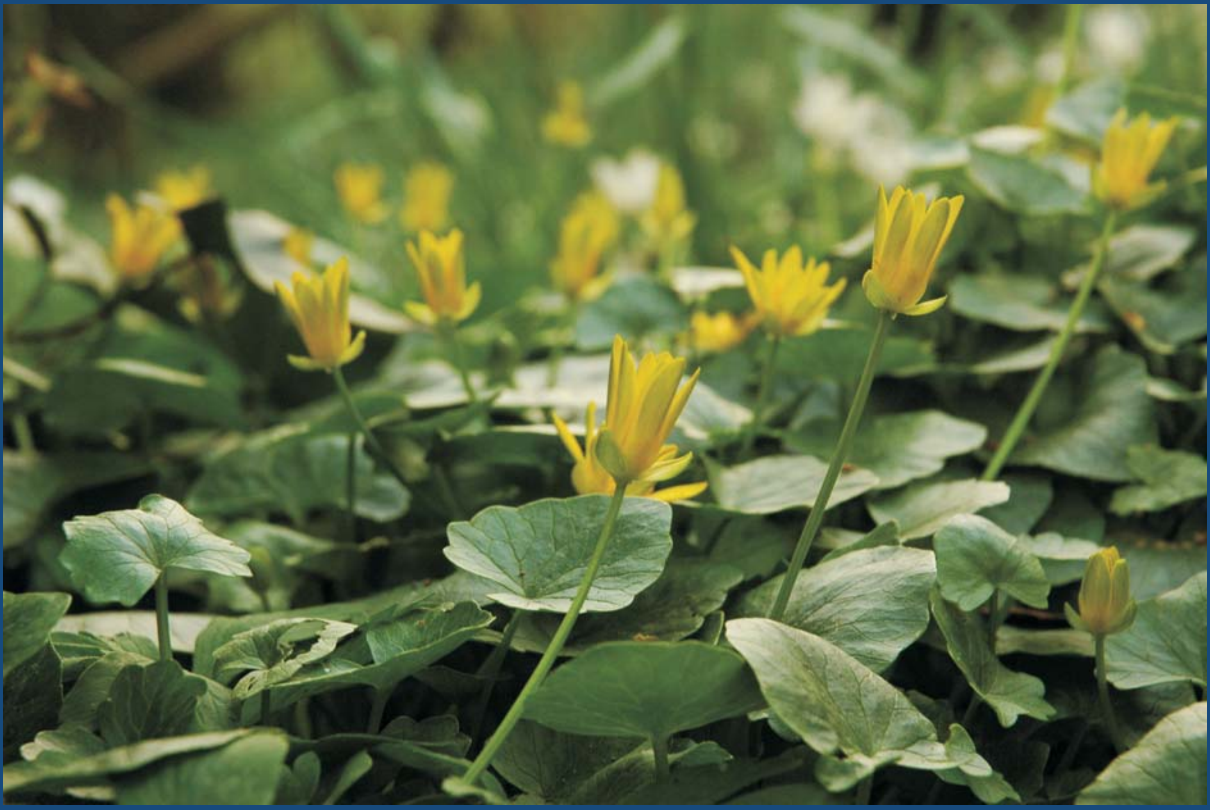


Recruitment and performance of forest understorey plants in post-agricultural forests

Lander Baeten

UNIVERSITEIT GENT

FACULTY OF BIOSCIENCE ENGINEERING

Supervisors: Prof. dr. ir. Kris Verheyen
Department of Forest and Water Management,
Laboratory of Forestry

Prof. dr. Martin Hermy
Katholieke Universiteit Leuven,
Department of Earth and Environmental Sciences,
Division Forest, Nature and Landscape

Dean: Prof. dr. ir. Guido Van Huylenbroeck

Rector: Prof. dr. Paul Van Cauwenberge

Lander Baeten

RECRUITMENT AND PERFORMANCE OF FOREST UNDERSTOREY PLANTS IN POST-AGRICULTURAL FORESTS

Thesis submitted in fulfillment of the requirements
for the degree of Doctor (PhD) in Applied Biological Sciences:
Land and Forest Management

Dutch translation of the title:
Rekrutering en groei van bosplanten in bossen op voormalige landbouwgronden

Illustrations on the cover:
Front: The fast colonizing forest plant *Ranunculus ficaria*, parc naturel des Hauts-Pays [photograph Karen Wuyts, May 2007]
Back: The slowly colonizing forest plant *Primula elatior*, la forêt domaniale du Tournibus [photograph Karen Wuyts, April 2008]

Citation: Baeten L (2010) Recruitment and performance of forest understorey plants in post-agricultural forests. PhD thesis, Ghent University, Ghent, Belgium.

ISBN-number: 978-90-5989-405-1

WOORD VOORAF

“Du choc des idées jaillit la lumière”, vertelde mijn grootvader mij vroeger wel eens. Wat ik toen niet verstond, ben ik jaar na jaar beter beginnen begrijpen. Vier jaar doctoraatsonderzoek is vier jaar van gedachten wisselen, discussiëren en leren van de ervaring van anderen. Een doctoraat maak je niet alleen, het is het resultaat van veel heen en weer pingpongen van ideeën. Twee promotoren, elk met hun eigen stijl, hebben mij vanaf mijn thesis op aanstekelijke manier gegidst. Kris, je enthousiasme voor wetenschappelijk onderzoek werkt enorm motiverend. Je hebt me wegwijs gemaakt in de ecologie van bossen, maar hebt vooral mijn blik op onderzoek enorm verruimd. Bovendien heb ik van jou altijd de kans en ruimte gekregen om ook buiten mijn doctoraat verschillende projecten aan te pakken. Martin, bedankt om je ervaring te delen, om je promotorschap zo geëngageerd op te nemen en om altijd alternatieve inzichten aan te geven. Je hebt me altijd gemotiveerd als student en beginnend onderzoeker. Bedankt allebei!

Gontrode is een memorabele plaats om te werken. Er zijn de constructieve discussies en de samenwerking tussen iedereen, maar ook de traktaties, de open haard, de buitenlandse excursies en de creatieve workshops die gaan van herbaria verbeteren tot duizenden bomen planten op een verzopen veld. Een dikke merci voor alle huidige en vroegere collega’s en zeker voor Christel, Luc, Greet en Kris die altijd een grote praktische hulp zijn. De rechterkant, altijd trouw op post en altijd bereid tot reciproque altruïsme. Bedankt voor je kritische blik naar mijn doctoraat, het noeste terreinwerk, het tolereren van zoveel onzin en de onophoudelijke stroom overleefpakketten.

Maar ook buiten Gontrode zijn er een aantal mensen te bedanken. Hans, je hebt tijdens mijn thesis de basis gelegd voor mijn verdere interesse in onderzoek. Een intensieve veldcampagne in Tournibus, de zoektocht naar geavanceerde statistische technieken en je kritische manier van onderzoek doen, gaven mij een vliegende start. Luc, bedankt voor het delen van je enorme terreinkennis in Tournibus, Muizenbos en andere bossen en de boeiende discussies. Eric, zonder jouw inzet voor de introductie-experimenten in Muizenbos en Leuven was een groot deel van dit onderzoek niet mogelijk geweest. Ook vijf thesisstudenten hebben mij hard geholpen bij veldwerk allerhande. Bedankt Wim, Tine, Sander, Pieter en Kathleen.

Furthermore, I thank all members of the jury for reading my manuscript and being a member of my PhD committee: Prof. Dries Bonte (Ghent University), Prof. Karel De Schamphelaere (Ghent University), Prof. Martin Diekmann (Bremen University), Prof. Ove Eriksson (Stockholm University) and Prof. Colin Janssen (Ghent University). Martin and Ove, thank you for coming all the way from Germany and Sweden!

Het Agentschap voor Innovatie door Wetenschap en Technologie financierde dit doctoraat gedurende vier jaar.

Er is natuurlijk niet alleen een “choc des idées” nodig. Dit doctoraat is er alleen maar omdat ik vanuit mijn omgeving altijd aangemoedigd en gesteund ben. Vake en moeke, de verwondering voor de natuur heb ik van geen vreemden en de kritische manier van in het leven te staan evenmin. Jullie hebben ons een prachtige jeugd meegeven vol ervaringen en herinneringen. Hiervoor kunnen we jullie nauwelijks genoeg bedanken! Sarah, Katrijn, Francisco, Johan, Jonathan, David, Dirk en Annie: bedankt voor jullie steun, maar vooral voor al de rest. De Guido’s, de Sint-Michielers en de land en bossers, merci voor alle schitterende momenten. Karen, bedankt voor je opgewektheid, je gedeelde interesses, je geduld, je bevestigingen… *throw those curtains wide*!

CONTENTS

SUMMARY

The distribution and abundance of forest understorey plant populations has been strongly influenced by human land-use history. Current and historical forest management may largely affect the understorey diversity, but the most radical impact probably comes from past agricultural land use. In forests growing on abandoned agricultural land, many species fail to (re)colonize after the forest vegetation was completely removed during the period of cultivation. Dispersal limitation initially causes the low colonization capacity of many forest understorey plant species, but low establishment rates may additionally reduce colonization success. Establishment limitation may arise if the environmental legacies of the former agriculture impede (stages of) plant recruitment or reduce plant performance. In this thesis, we studied whether plant recruitment and performance are influenced by former agricultural land use with particular attention for the direct and indirect effects of its fertilization legacy.

The **recruitment** rates of ten different forest plant species in post-agricultural valley forests and plateau forest were determined with a seed sowing experiment. The established vegetation and litter were cleared in half of the experimental plots. After two growing seasons, only a small fraction of the seeds had germinated (often < 5 %) and competition with the established vegetation significantly reduced the number of seedlings of non-vernal species in the valley forest sites. Post-seedling mortality further decreased the number of seedlings that effectively recruited into the adult life stage. Low recruitment rates may apparently limit the colonization success of forest plants, especially if high nutrient levels support a vigorously growing established vegetation. In a similar introduction experiment, we focused on two forest herbs and performed a detailed monitoring of their recruitment and longevity during eight growing seasons. Seeds and adults of the slowly colonizing *Primula elatior* and the fast colonizing *Geum urbanum* were introduced in post-agricultural and ancient forest sites. An important result of this experiment was that the longevity of both species was significantly lower in post-agricultural compared to ancient forest, which was partly an effect of strong competition, and that *P. elatior* could not counterbalance the lower longevity because of its low germination and low recruitment into the adult stage. A germination experiment indicated that the low germination of *P. elatior* in post-agricultural forest was not due to a low seed quantity or germinability in the experimental population; the past agriculture and P supply were even beneficial. Slowly colonizing species are thus not only limited by dispersal, but also by establishment if particular stages of recruitment are reduced in post-agricultural forest.

Once a forest plant has recruited, the former land use may affect its **performance**. We determined whether a soil nutrient legacy of past agriculture (here N and P supply) is related to differences in plant performance between post-agricultural and ancient forest. Seven forest plants species with varying colonization capacity showed much higher tissue P concentrations in post-agricultural compared to ancient forest; the N concentrations were unrelated to past land use. Both N and P concentrations varied among species, but this variation was unrelated to their colonization capacity. Six species had a significantly higher biomass in post-agricultural compared to ancient sites and the degree to which the species increased their biomass was positively related to their colonization capacity. In a pot experiment with four forest herbs, we similarly showed that higher P supply increased tissue P concentrations and increased the vegetative performance of two species (*P. elatior* and *Circaea lutetiana*). Differences in generative performance were rather related to differences in light supply. In sum, we showed that an agricultural soil nutrient legacy, and P availability in particular, may be beneficial for plant performance, but with the fastest colonizers potentially showing a stronger response.

If dispersal alone limits the colonization of forest plants, species diversity in post-agricultural forests will gradually increase over time and the community composition will converge to ancient forest vegetation. In a vegetation resurvey, we found no evidence for such community development after 30 years. In fact, strong community changes in both ancient and post-agricultural forest were associated with an increase of common, competitive species, which might provoke stronger establishment limitation. Moreover, as populations of ancient forest indicator species declined, source populations were depleted and dispersal limitation might have increased as well.

Our results provide insight into the effect of past land use and its fertilization legacy on the recruitment and performance of forest understorey plants. While some stages of plant establishment are enhanced in post-agricultural compared to ancient forest, others are rather reduced. The latter stages may form important bottlenecks for plant colonization.

SAMENVATTING

De landgebruiksgeschiedenis van bossen beïnvloedt de verspreiding en abundantie van bosplantenpopulaties. Het huidige en historische bosbeheer kunnen een grote invloed hebben op de kruidlaagdiversiteit, maar een voormalig landbouwgebruik heeft waarschijnlijk de meest verregaande impact. Veel bosplantensoorten kunnen bossen op voormalige landbouwgronden moeilijk (her)koloniseren. De kolonisatie wordt allereerst bemoeilijkt door de beperkte dispersiecapaciteit van vele soorten [E: *dispersal limitation*], maar beperkte vestigingskansen kunnen het kolonisatiesucces verder verminderen [E: *establishment limitation*]. Een beperkte vestiging kan veroorzaakt worden doordat het voormalige landbouwgebruik de omgevingscondities zodanig wijzigde dat de rekrutering wordt belemmerd of dat de groei van adulte individuen vermindert. In deze thesis onderzochten we of de rekrutering en groei van bosplanten beïnvloed worden door voormalig landbouwgebruik, met bijzondere aandacht voor de directe en indirecte effecten van vroegere bemesting.

Het **rekruteringssucces** van tien verschillende bosplantensoorten werd bepaald met een zaai-experiment in vallei- en plateaubossen op voormalige landbouwgrond. De gevestigde vegetatie en de strooisellaag werden in de helft van de experimentele plots verwijderd. Slechts een kleine fractie van de zaden was gekiemd na twee groeiseizoenen (vaak < 5 %) en competitie met de gevestigde vegetatie reduceerde het aantal zaailingen in valleibos (niet voor voorjaarssoorten). Mortaliteit na het zaailingstadium verminderde verder het aantal zaailingen dat effectief een adult levensstadium bereikte. Een laag rekruteringssucces kan dus de kolonisatie van bosplanten verhinderen, vooral indien de gevestigde vegetatie sterk profiteert van een hoge nutriëntenbeschikbaarheid en weelderig groeit. In een gelijkaardig introductie-experiment focusten we op twee soorten waarvoor de rekrutering en langlevendheid opgevolgd werden gedurende acht groeiseizoenen. De traag koloniserende soort *Primula elatior* (slanke sleutelbloem) en de snel koloniserende soort *Geum urbanum* (geel nagelkruid) werden geïntroduceerd als zaad en als adult individu in bos op voormalige landbouwgrond en in oud bos. Een belangrijk resultaat van dit experiment was dat de langlevendheid van adulten van beide soorten significant lager was in bos op voormalige landbouwgrond, ten dele door de sterke competitie met de gevestigde vegetatie, en dat *P.*

elatior niet kon compenseren voor deze lagere overleving door het gebrek aan veel nieuwe zaailingen en beperkte rekrutering tot een adult levensstadium. Een kiemproef toonde dat het beperkt aantal zaailingen van *P. elatior* in bossen op voormalige landbouwgrond niet te wijten is aan lage zaadkwantiteit of kiemkracht; voormalig landbouwgebruik en P-beschikbaarheid waren zelfs bevorderlijk. De trage kolonisatie van bosplanten is dus niet alleen te wijten aan beperkte dispersie, maar ook aan verminderde vestiging wanneer het voormalige landbouwgebruik bepaalde rekruteringsstadia belemmert.

Naast rekrutering kan het voormalige landgebruik ook de **groei** van een bosplant beïnvloeden. We bepaalden of een veranderde beschikbaarheid van bodemnutriënten door voormalig landbouwgebruik (hier specifiek N en P) gerelateerd is aan verschillen in de groei van bosplanten in bossen op voormalige landbouwgrond en oude bossen. Zeven bosplantensoorten met een verschillende kolonisatiecapaciteit hadden consistent hogere P-concentraties in hun bovengrondse biomassa op voormalige landbouwgrond; de N-concentraties waren onafhankelijk van het voormalige landgebruik. De N- en P-concentraties verschilden sterk tussen de zeven soorten, maar deze variatie was niet gerelateerd aan hun kolonisatiecapaciteit. Zes soorten hadden een hogere bovengrondse biomassa in de bossen op landbouwgrond en de mate waarin ze hun biomassa verhoogden was positief gerelateerd aan hun kolonisatiecapaciteit. In een potexperiment met vier bosplantensoorten vonden we ook dat hogere P-beschikbaarheid leidde tot hogere P-concentraties in de biomassa van de bestudeerde soorten en tot betere vegetatieve groei voor twee van de vier soorten (*P. elatior* en *Circaea lutetiana*, groot heksenkruid). Verschillen in regeneratieve groei waren grotendeels het gevolg van verschillen in lichtbeschikbaarheid. We toonden aan dat een veranderde nutriëntenbeschikbaarheid door voormalig landbouwgebruik, and P-beschikbaarheid in het bijzonder, de groei van bosplanten kan bevorderen, maar de snel koloniserende soorten vertonen mogelijk de sterkste respons.

Wanneer de kolonisatie van bosplanten enkel belemmerd wordt door hun beperkte dispersiecapaciteit zal de kruidlaagdiversiteit in bossen op voormalige landbouwgrond gradueel toenemen gedurende de bosontwikkeling. Bovendien zal de samenstelling van de kruidlaag steeds meer die van oud bos benaderen. In een heropnamestudie vonden we echter geen aanwijzingen voor een dergelijke kruidlaagontwikkeling over een periode van 30 jaar. Zowel in oud bos als in bossen op voormalige landbouwgrond veranderde de samenstelling van de kruidlaag sterk, o.a. door een toename van algemene, competitieve soorten die de vestigingskansen voor oudbosplanten verder kunnen reduceren. Bovendien gingen de populaties van oudbosplanten sterk achteruit zodat bronpopulaties verdwenen en de dispersiemogelijkheden verder beperkt werden.

De resultaten in deze thesis bieden inzicht in de impact van voormalig landbouwgebruik en de vroegere bemesting op de rekrutering en groei van bosplanten. Sommige stadia in de vestiging van planten worden bevorderd door het voormalige landgebruik, andere worden eerder belemmerd. Het zijn deze laatste stadia die sterke knelpunten vormen voor de kolonisatie van bosplanten.

LIST OF ABBREVIATIONS, SYMBOLS AND DEFINITIONS

Abbreviations and symbols

β_{RC}	presence-absence based community divergence
Ca^{2+}	calcium
CCI	colonization capacity index
DCA	detrended correspondence analysis
d.f.	degrees of freedom
E	evenness index
F_{ST}	cover based community divergence
GLMM	generalized linear mixed model
K^+	potassium
Mg^{2+}	magnesium
n	sample size
N	nitrogen
P	phosphorus
P	significance of statistical test
PCA	principal component analysis
PPI	plant performance index
r	correlation coefficient
R^2	coefficient of determination
SE	standard error
SD	standard deviation
SR	species richness

Nomenclature

The International Plant Names Index (2010). URL http://www.ipni.org [accessed August 2010]

Definition of some terms used in the thesis

colonization capacity	the ability of plants to establish a population in unoccupied post-agricultural forest, i.e., species that are strongly associated with ancient forest have a low colonization capacity
dispersal limitation	the distribution of species is restricted by seed dispersal, i.e., adding seeds to unoccupied habitats results in successful germination and establishment

establishment limitation	plant population size is constrained by the environmental conditions (not by the number of seeds), which restrict one or more stages of plant recruitment after seed deposition or reduce adult performance
recruitment	the transitions in plant demography from seed germination until the growth into the adult life stage

CHAPTER 1 – LAND-USE HISTORY AND THE DISTRIBUTION OF FOREST UNDERSTOREY SPECIES IN TEMPERATE FOREST LANDSCAPES

One of the central challenges in plant ecology is explaining the distribution and abundance of plant populations in space and time. The complexity of this seemingly simple issue is illustrated by the vast number of theories and their underlying assumptions that are devoted to it (e.g., MacArthur & Wilson 1967; Tilman 1982; Grime 2001; Hubbell 2001; Chase & Leibold 2003; Craine 2005; Vellend 2010). The present thesis studies the basic ecological question how past disturbances (*sensu* Picket & White 1985) versus current environmental conditions influence plant distribution and abundance in present-day landscapes. Disturbances and environmental conditions play a pivotal role in classical vegetation ecology (e.g., Pickett & White 1985; Grime 2001), and they are subject to pervasive human impacts. Understanding how disturbance and environmental conditions structure plant communities is therefore crucial if we want to assess the increasing human influence on vegetation.

The most radical and extensive human disturbance in much of Europe and eastern North America over the past centuries was probably the conversion of forests into agricultural land, followed by the abandonment of agriculture and subsequent forest recovery on this set-aside land (Whitney 1994; Kirby & Watkins 1998; Honnay et al. 2004). Generally, the former agricultural use has not only removed the local forest (understorey) plant populations, but also modified the environmental conditions. The former land use, i.e., land-use history, may thus influence the distribution and abundance of forest plant populations both directly, through disturbance, and indirectly, through its impact on the (a)biotic environment (reviewed in: Hermy et al. 1999; Honnay et al. 2004; Flinn & Vellend 2005; Gilliam 2007; Hermy & Verheyen 2007). Below, I will briefly outline (§1) how the past and future land-use changes created or may create complex forest landscapes, (§2) how the agricultural history may have altered the forest environmental conditions and (§3) how dispersal and establishment limit the (re)colonization of forest understorey plants after a past disturbance. This last part summarizes the current state of knowledge regarding the mechanisms that underlie the patterns of forest plant colonization and identifies particular research gaps. The concluding section (§4) translates the research gaps into to the main objectives and structure of the thesis. Obviously, the present chapter should not be read as an exhaustive review, but rather as a concise introduction that places the thesis into a broader context.

1 Land-use change: historical perspective and future directions

Much of the current forest area in western Europe and eastern North America has been profoundly influenced by human activities for many centuries to even millennia (e.g., Peterken 1993; Whitney 1994; Foster et al. 1998; Kirby & Watkins 1998; Rackham 2003). In northern Belgium (Flanders region), detailed historical ecological research has found clear (pre)historical evidence of forest use by humans for many centuries (e.g., Tack et al. 1993; Verheyen et al. 1999; Baeté et al. 2009). The historical use of forests includes a wide variety of management practices such as traditional coppicing, charcoal production, cattle grazing and litter removal. Yet, the reclamation of forests for agriculture is by far the most intensive human impact on forests.

Large-scale deforestation of temperate forests for agriculture and subsequent forest recovery after agricultural abandonment have been well documented in both eastern North America and western Europe. In eastern North America, the conversion of forests into agricultural land, mainly by European colonists, largely occurred during the 18th and 19th century (e.g., Foster et al. 1998; Hall et al. 2002; Smith et al. 2004; Flinn & Vellend 2005). Since the beginning of the 20th century, the forest area has gradually increased again. Land-use history is therefore relatively straightforward: it includes a single deforestation phase followed by a single phase of forest recovery. As an example, Foster et al. (1998) found that forest cover in central Massachusetts was > 99 % in the mid-17th century, but rapidly decreased to 20–30 % in the mid-18th and 19th century. During the 20th century, massive areas of agricultural land were abandoned and the forest area increased again to 80–90 %.

In northwestern Europe, however, landscape dynamics are usually far more complicated. First of all, the first deforestations for agriculture occurred many centuries ago (Tack et al. 1993; Verheyen et al. 1999; Rackham 2003). For example, clear evidence of Roman agriculture almost 2000 years ago was found in present-day forests in northern and central

France (Dupouey et al. 2002; Dambrine et al. 2007; Plue et al. 2008) and evidence of deforestation goes back as far as the early Neolythic times (Tack et al. 1993). Second, the current forest area is often the result of several phases of deforestation and reforestation. In the western part of Belgium (the former county of Flanders), for instance, Tack et al. (1993) documented four major periods of forest regression and forest expansion during the past 6000 years. Therefore, present-day forests often consist of a mosaic of stands with contrasting land-use history. This is nicely illustrated by the forest 'Muizenbos' (central northern Belgium), which is used as a study area in three chapters of this thesis (Fig. 1.1). Third, the deforested area has generally been quite extensive, but only parts of this area have become forested again afterwards. Consequently, the current forest area does not attain the pre-clearance levels and complex landscapes with scattered forest patches of highly variable age have developed. In northern Belgium (Flanders region), forests cover only 10.8 % of the total land area. An analysis of cartographical data from 1775 to 2000 showed that only 15 % of this area (c. 23500 ha) has been continuously forested since the end of the 18th century (De Keersmaeker et al. 2001).

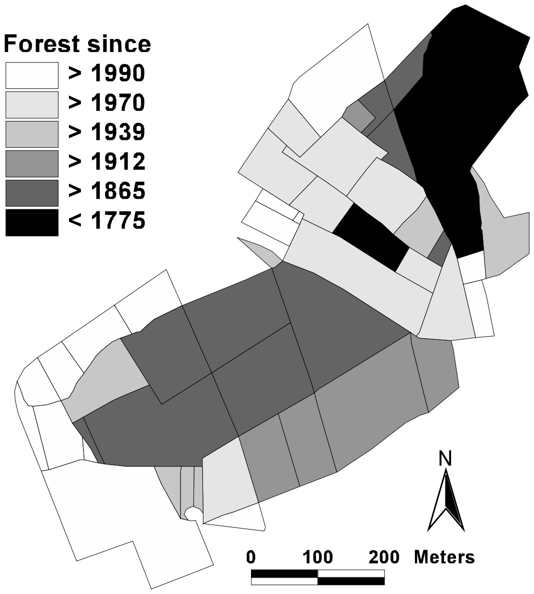


Fig. 1.1 Age of the different forest stands in the forest 'Muizenbos' (Ranst, Belgium). The youngest stands were afforested just after 1990; the oldest stands have been forest at least since the oldest land-use map (De Ferraris 1775). This map was based on data from Verheyen (2002).

Land use change is also a topical subject, seeing that the total European forest area increased with 0.9 mio ha.year^{-1} between 1995–2000 and 0.66 mio ha.year^{-1} between 2000–2005 (FRA 2005). Furthermore, intentions for future forest expansion are expressed in a number of European political programmes (Weber 2000), e.g., as a measure to reduce nitrogen pollution (Nitrogen directive 91/676/EEC) and as a sink for carbon dioxide (Kyoto

Protocol, Convention on Climate Change). The Environmental Policy Plan for Flanders formulates a target of 10 000 ha ecologically sound forest expansion (Mina-plan 3; Natuurindicatoren 2006).

The ubiquitous human influence on forests in northwestern Europe implies that we have lost all '**virgin forest**', i.e., natural woodland which has never been significantly influenced by people (Peterken 1996). However, despite the overall impact of human activities, we may discern between '**primary forest**', i.e., forests that have existed continuously since before the original post-glacial forests in the region were fragmented and '**secondary forest**', i.e., forests that have originated on unwooded sites ever since (Peterken 1996; Rackham 2003; see Fig. 1.2). The terms primary and secondary thus refer to forest continuity and not to stand structure and function. Because agricultural use may pre-date the historical records in most of Europe and the status of a primary forest is difficult to define, forests are pragmatically classified as '**ancient forest**', i.e., forests that have been continuously forested at least since the oldest available historical data, or '**post-agricultural forest**', i.e., forests that have (been) established on former agricultural land after that date (Peterken 1996; Rackham 2003). The terms ancient and post-agricultural will be consistently used in this thesis. Although in the strict sense both terms only refer to forest continuity, we will use them to indicate important differences in land-use history. In this thesis, post-agricultural forests are relatively recent afforestations on land that has been cultivated for several years, i.e., ploughed and fertilized. They have (been) established during recent decades, after a certain threshold date (Fig. 1.2). Ancient forest sites, on the other hand, have not been used for agriculture since the same threshold date, e.g., 1775 (Ferraris Map) for Belgium (Fig. 1.2). Traditional forms of agricultural use at some point in the history of these ancient forests cannot be excluded (e.g., Tack et al. 1993; Dupouey et al. 2002; Dambrine et al. 2007; Plue et al. 2008), but those practices, if any, pre-date the time of agricultural intensification. The possible legacies of former agriculture on the soil and vegetation are therefore much smaller in ancient compared to post-agricultural sites (see §2).

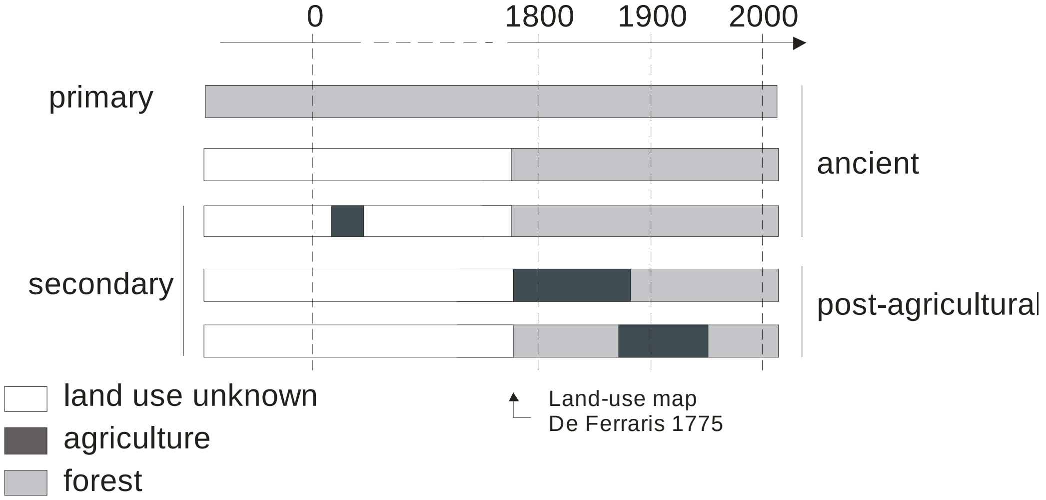


Fig. 1.2 Illustration of the different terms that are used to describe the land-use history of forest sites. The terms ancient and post-agricultural forest will be consistently used in this thesis. See text for further explanation.

2 The environmental legacies of former agriculture

If we want to understand the distribution and abundance of forest plants in post-agricultural versus ancient forests, it is crucial to assess the legacies of past agriculture on the present-day habitat quality. The impact of the former land use basically involves the alteration of the (a)biotic soil conditions and the canopy structure and composition. There is strong evidence that other human impacts such as the alteration of the management regime and atmospheric deposition additionally change the habitat quality in forests, but their impact on the vegetation in both ancient and post-agricultural forest will not be discussed here (reviews: Gilliam 2006, 2007; Rackham 2008; Baeten et al. 2010).

2.1 Forest soil

Agricultural land use has an enormous impact on the physico-chemical soil conditions, which outlasts the duration of the agricultural activities (McLauchlan 2006). This implies that soil differences between post-agricultural and ancient forest sites may be important. Yet, the impact of agriculture may differ considerably among sites and particular chemical soil properties.

Variation among sites – Although the magnitude and persistence of the soil legacies may vary largely among sites (Table 1.1), post-agricultural forest soils generally have higher pH values and nutrient concentrations and a lower organic matter content compared to ancient forest soils (Flinn & Vellend 2005). The different impact of former agriculture between sites may be largely explained by the particularities of the past agricultural management practices. First, as the increased nutrient levels and pH mainly originate from the application of fertilizers, the duration of the agricultural use seems pivotal. Honnay et al. (1999) and Verheyen et al. (1999), for instance, found a positive correlation between the duration of the arable land use and the pH and the soil phosphorus (P) and calcium concentrations. Second, agricultural practices have intensified over time, especially during the last decades. This implies that the differences in chemical soil properties between ancient forest sites and agricultural land upon its abandonment will be much higher at present compared to several decades ago. De Keersmaeker et al. (2004) found similar total soil P concentrations in ancient forest stands and 100–150 year old afforestations, but much higher P levels in forests younger than 50 years. The period of agricultural intensification may differ considerably among regions. Several studies, mainly in western Europe, showed that early to mid-19th century afforestations clearly bear the imprint of the former agricultural use (e.g., Koerner et al. 1997; Honnay et al. 1999; Verheyen et al. 1999), while others, mainly in North America, could not find strong evidence of past fertilization (e.g., Bellemare et al. 2002; Flinn et al. 2005; Fraterrigo et al. 2005). Third, the magnitude of the soil legacies depends on the type of former cultivation, especially grassland versus cropland management (Koerner et al. 1997; Compton & Boone 2000). In former grasslands, for instance, the reduction in organic matter content and the increase in nutrient concentrations are generally smaller.

Variation among soil properties – The magnitude, direction and persistence of the agricultural soil legacies also vary considerably among the different chemical soil properties (reviewed in McLauchlan 2006; Table 1.1). Former fertilization may have increased the soil concentrations of base cations (K^+, Ca^{2+} and Mg^{2+}). However, those elements are depleted relatively fast (time scale of decades) because of leaching and the accumulation in biomass during forest growth (Richter et al. 1994). Differences in soil acidity between ancient and post-agricultural sites may therefore gradually decrease in a time span of decades (Richter et al. 1994; Bossuyt et al. 1999a; Matlack 2009), but it may also take up to a century or more (Koerner et al. 1997; Verheyen et al. 1999; Falkengren-Grerup et al. 2006). The soil organic carbon generally decreases fast after the conversion of forest into agricultural land because of reduced annual carbon input (harvests), increased decomposition rates of the organic matter and erosion (Davidson & Ackerman 1993; McLauchlan 2006). The organic carbon reaccumulates during forest growth after land abandonment (e.g., Richter et al. 1994; Hooker & Compton 2003; Richter & Markewitz 2001; Vesterdal et al. 2002; Matlack 2009), but, again, it may take several decades for the organic carbon content to become similar to the levels of nearby ancient forest sites (e.g., Falkengren-Grerup et al. 2006; Valtinat et al. 2008).

Table 1.1 Non-exhaustive overview of studies that compared the chemical soil properties of post-agricultural and ancient forest sites: pH, base cations (BC), total organic matter/total carbon (OM), nitrogen (N) and phosphorus (P) concentrations. The symbols indicate higher (+), equal (0) or lower (–) values in post-agricultural sites, respectively. Chronosequences were not included. If a study reported more than one soil depth, the results for the upper soil layer were included here. The same holds for different periods of abandonment; only one period was chosen. Extraction methods for N and P differed considerably among studies, ranging from the total pool to concentrations in the soil solution.

Location	Soil	Year of abandonment	pH	BC	OM	N	P	Reference
a) western Europe								
Tronçais forest, France	sand	~4th century	+		0	0	+	Dambrine et al. 2007
Lorrain, France	silty clay, clay	~4th century	+		+	+	+	Dupouey et al. 2002
Compiègne forest, France	heterogeneous	~4th century	+	0	0	0	+	Plue et al. 2008
central Zealand, Denmark	sand	19th century	0		0		0	Graae et al. 2003
Jura Mountains, France	clay, clay loam, loam	> 1830	0	0	0	0	0	Sciama et al. 2009
Vosges Mountains, N France	(no data)	1880-1936	+		-/0/+	0/+	+	Koerner et al. 1997
Ename forest, Belgium	sandy loam	> 1884	+	0/+	0	-	0/+	Verheyen et al. 1999
Skåne province, S weden	(no data)	1920-1950	+		-	0	+	Valtinat et al. 2008
Skåne province, S weden	(no data)	1920-1960	+		-	-	+	Falkengren-Grerup et al. 2006
central and southern Finland	sandy loam, loam	1930-1940	+	0/+	+	+	+	Wall & Hytönen 2005
Doupovské Mountains, Czech Republic	(no data)	> 1953	0		-	-	0	Kopecký & Vojta 2009
Muizenbos forest, Belgium	loamy sand, sandy loam	> 1970	0		-	-	+	De Keersmaeker et al. 2004
b) eastern North America								
western Massachusetts	sandy loam	1830-1942	0	0	0	0	0	Bellemare et al. 2002
Harvard forest, Massachusetts	loam	1875-1905			0	+	+	Compton & Boone 2000
Tompkins County, New York	silt loam	1900-1938	+	0	0	0	0	Flinn et al. 2005
Tompkins County, New York	silt loam	1900-1938	0	0	-	0	-	Flinn & Marks 2007
Montague Plain, Massachusetts	sand	1900-1960	0	-/0	-			Motzkin et al. 1996
southern Appalachian forests, North Carolina	(no data)	1930-1975	0	0	0	0	0	Fraterrigo et al. 2005

The magnitude, direction and persistence of the impact of the former agriculture on the available pools of soil nitrogen (N) and phosphorus (P) are of particular interest because N and P represent essential resources for plant growth (Marschner 1995; Aerts & Chapin 2000; Güsewell 2004; Craine 2009). The dominant paradigm is that the fate of these two nutrients in the soil differs markedly during forest recovery. Richter & Markewitz (2001) state that of all plant nutrients, N is the one capable of changing very substantially in soils of aggrading forest ecosystems over time scales of only decades. This has important implications for the persistence of land-use effects on soil N. Agricultural use generally depletes the total N pool through multiple loss pathways such as decomposition of organic matter and leaching (McLauchlan 2006). As forest re-establishes, the vegetation takes up large amounts of N, so that the topsoil available N is further depleted (Richter & Markewitz 2001; Hooker & Compton 2003; reviewed in Hansen et al. 2002). Due to the gradual accumulation of organic matter, the total soil N will increase again, but this may take centuries (Verheyen et al. 1999). Despite the effects of former land use on the total soil N content, plant-available fractions may not be affected perceptibly (for plants). When the input of inorganic N (e.g., from litter decomposition and N deposition) exceeds the demand of microorganisms and plants, the surplus leaves the system through soil leaching (Aber et al. 1989). During the first two decades of forest recovery on former agricultural land, Hansen et al. (2007) found negligible nitrate leaching due to high vegetation N uptake (cf. Richter & Markewitz 2001). After canopy closure, however, the decreased N demand by the vegetation, increased litter inputs and increased N deposition led to N leaching. Thus, from a plants point of view, land-use effects on the total available N may not be perceived because of its abundance (i.e., not limiting resource) in both ancient and post-agricultural forest.

In contrast to N, residual effects of P fertilization persist because of the close coupling of decomposition and uptake, the strong geochemical fixation on calcium, iron and aluminium minerals and the lower number of input and loss pathways (Wood et al. 1984; McLauchlan 2006; Richter et al. 2006). When manure is applied to meet the optimal N requirements of crop plants, P is often in excess and, due to its persistence in the soil, P will accumulate every year. In forests established on former agricultural land, this P legacy may persist for centuries to even millennia (see Table 1.1). The bioavailable P pool, which is typically only a fraction of the total pool, also remains high because the uptake from this pool in biomass is continuously replenished from other pools (Richter et al. 2006). The potential role of P enrichment for community recovery will be discussed below (§3.2 – Resource alterations).

Spatial homogenization – Land-use change may not only alter the overall levels of the different chemical soil properties, it also affects their spatial heterogeneity. Fraterrigo et al. (2005) and Flinn & Marks (2007) showed that former agriculture may homogenize the spatial structure of soil N, P, C and pH. The homogenization is mainly caused by the forest clearing itself, soil tillage and the uniform inputs of soil amendments. It remains largely to be studied, however, how this homogenization may affect the recovering plant population.

2.2 Forest canopy

If forests are left to recover spontaneously on abandoned land, the species composition and the structure of the tree and shrub layer of these new forests may differ considerably from forests that are already wooded for a long time. Several studies in eastern North America found strong differences between the tree communities of primary forests (i.e., selectively cut but continuously wooded) and forests that established more than a century ago on agricultural land (Foster et al. 1998; Bellemare et al. 2002; Flinn & Marks 2007). Differences in the species composition and structure of the canopy may be associated with differences in light availability at the forest floor and differences in the quantity and quality of the litter layer (e.g., Verheyen & Hermy 2001a; Gilliam 2007). Those environmental factors may in turn affect the understorey community composition (e.g., Sydes & Grime 1981; van Oijen et al. 2005; Van Calster et al. 2008a). In much of western Europe, however, forest management has a dominant impact on the composition and structure of the forest canopy layer and this layer may not consistently differ between stands with contrasting land-use history. In both post-agricultural and ancient stands, the shrub and tree layer did generally not develop spontaneously, but are the result of active tree planting and intensive forest management. Land-use change *per se* is therefore not the determinant factor for the overstorey community development (Verheyen & Hermy 2001a) and, in fact, the way a site was afforested or replanted (e.g., tree species used, planting a shrub layer or not) may influence the understorey community much more (De Keersmaeker et al. 2004).

3 Colonization of forest understorey plants: a multi-stage process

After agricultural abandonment and forest recovery, forest understorey species need to colonize from remnant source populations distributed across the landscape. Forest plants differ, however, remarkably in their capacity to colonize post-agricultural forest patches. This was already acknowledged several decades ago (e.g., Roisin & Thill 1953; Froment & Tanghe 1967), but was first studied in detail by George Peterken in 1974. In his seminal paper about plant distribution in forest patches across central Lincolnshire (UK), Peterken (1974) discriminated between slowly colonizing species that were strongly confined to ancient forest (‘**ancient forest species**’ *sensu* Hermy et al. 1999) and species that showed a higher, but varying colonization rate into post-agricultural sites. Since then, a number of other authors have worked out a similar species classifications for different landscapes in Europe (e.g., Belgium: Hermy & Stieperaere 1981, Honnay et al. 1998; Denmark: Petersen 1994, Graae 2000; France: Sciama et al. 2009; Germany: Wulf 1997, 2003; Poland: Dzwonko & Loster 1989) and eastern North America (e.g., Whitney & Foster 1988; Matlack 1994; Motzkin et al. 1996; Singleton et al. 2001; Bellemare et al. 2002). Currently, there is scientific consensus that plant species distributions bear the imprint of former land use for many centuries because a suite of understorey species fail to (re)colonize during forest development on former agricultural land (reviewed in Hermy et al. 1999; Verheyen et al. 2003c; Honnay et al. 2004; Flinn & Vellend 2005; Hermy & Verheyen 2007; Vellend et al. 2007).

Starting from the observation that species differ in their colonization capacity, research has moved on to *explaining why the colonization capacity of species might differ*. Basically two non-mutually exclusive hypotheses have been put forward (Fig. 1.3; Eriksson & Ehrlén 1992; Clark et al. 2007). First, slowly-colonizing species are dispersal limited, i.e., no or an insufficient amount of diaspores arrive at the new habitat patches to form a founding population (‘**dispersal limitation**’). Second, the former land use has modified the environmental conditions in a way that restricts one or more stages of plant establishment, including germination, recruitment from the seedling into the adult stage and adult performance (‘**establishment limitation**’). Assessing the relative importance of both hypotheses is not only relevant within the context of recovering forest plant communities, but also contributes to the broader ecological question as to how habitat suitability and dispersal structure plant communities. Both dispersal and establishment limitation will be (briefly) discussed below.

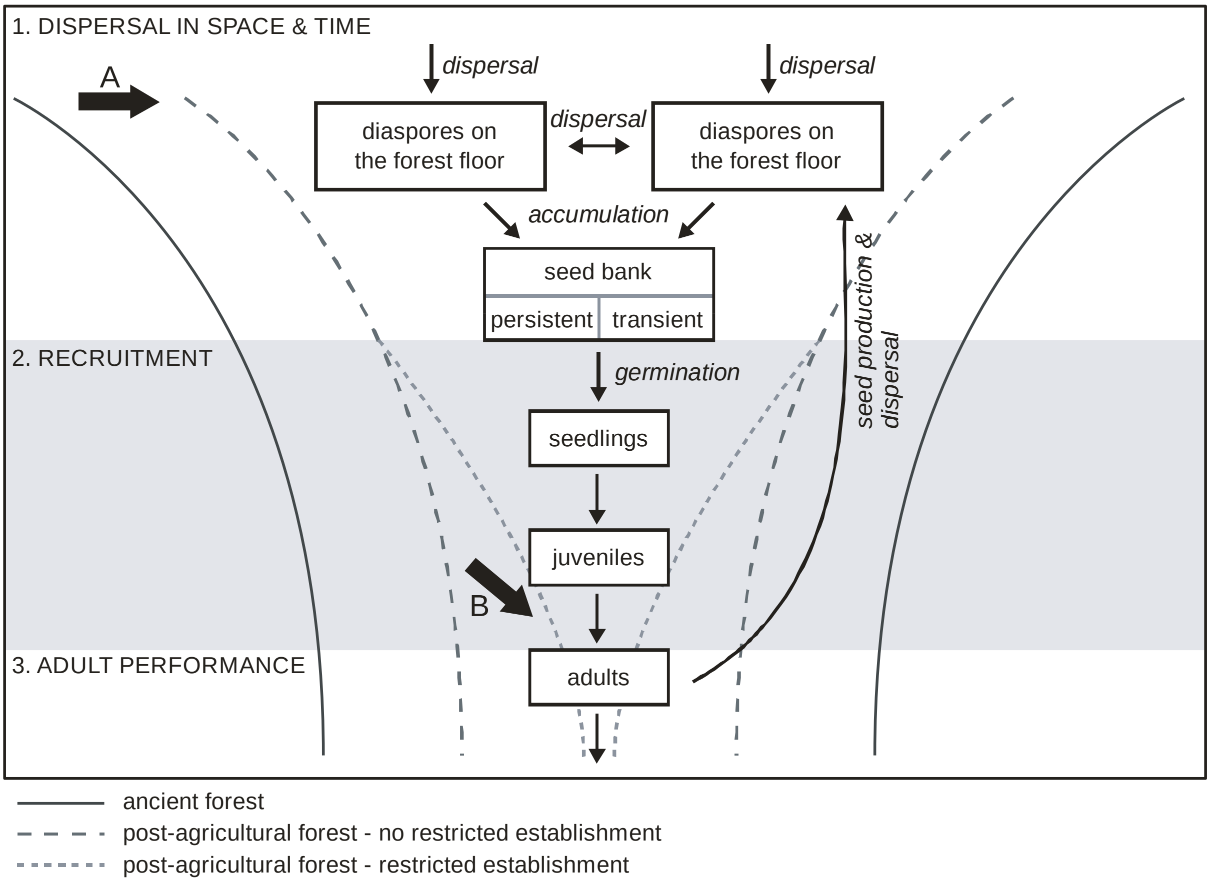


Fig. 1.3 Conceptual bottleneck model of plant colonization in post-agricultural and ancient forest. The funnel-shaped lines represent the decreasing number of individuals through the different life stages, due to mortality and predation. Dispersal limitation (arrow A) causes lower diaspore availability in post-agricultural forest. Establishment limitation, caused by altered environmental conditions, may narrow the funnel even more (arrow B). The strength of the environmental effect may be species specific and is highly dependent on the nature of the former land use (adapted from Hermy & Verheyen 2007).

3.1 Dispersal limitation

Three types of studies have provided evidence that dispersal limitation primarily hampers forest plant colonization: space-for-time studies, life-history approaches and seed sowing experiments.

Spatio-temporal isolation – If the low colonization capacity of forest plants is controlled by limited dispersal, the frequency and abundance of these species in post-agricultural forest patches will increase with increasing forest age and decreasing isolation from colonization sources. In chronosequence studies, the forest plant species richness indeed increased with forest age (e.g., Bossuyt et al. 1999b; Jacquemyn et al. 2001; Flinn & Marks 2004; Brunet 2007). Furthermore, at the landscape scale, several studies found a negative effect of isolation on the forest understorey richness or species occurrence in post-agricultural forest patches (e.g., Peterken & Game 1984; Matlack 1994; Grashof-Bokdam & Geertsema 1998; Jacquemyn et al. 2001; Honnay et al. 2002b). The importance of the distance to colonization sources was also clearly demonstrated in ecotone studies, which describe vegetation patterns along transects from ancient into adjacent post-agricultural stands. Within the post-agricultural stand, species occurrence and abundance generally decrease with distance from the ancient forest boundary (Matlack 1994; Brunet & von Oheimb 1998; Bossuyt et al. 1999b; Donohue et al. 2000; Singleton et al. 2001; Orczewska 2009). Ecotone studies were also used to calculate species-specific colonization rates, which generally ranged between 0–2 m.year^{-1}. Finally, detailed species mapping at the forest (Verheyen & Hermy 2001b) and landscape scale (Verheyen et al. 2003b) were used to demonstrate the integrated effects of both forest age and spatial isolation from species-specific colonization sources. Collectively, this overwhelming amount of evidence confirms that dispersal is a major driver of forest understorey species colonization.

Life-history traits – If species with a contrasting colonization capacity consistently differ in a suite of life-history traits, these traits may partly account for the variation in plant colonization rates. The majority of studies only looked at the average migration rates among species groups with different dispersal modes. While a number of studies found higher migration rates for species dispersed by vertebrates (endo/epizoochory) or wind compared to species dispersed by ants or without adaptations for an external dispersal vector (Matlack 1994; Brunet & von Oheimb 1998; Grashof-Bokdam & Geertsema 1998), this observation was not universal. Hermy et al. (1999), for instance, reviewed 22 papers and showed that a considerable proportion of the ancient forest species can in fact be dispersed by vertebrates (27 %) or wind (25 %). Graae & Sunde (2000) and Verheyen et al. (2003c) performed a more profound screening of traits and found that slowly-colonizing species are generally characterized by a lower dispersability (e.g., heavy seeds, low fecundity, short distance dispersal vectors, high extent of lateral spread). Together, the results of the studies on life history traits point to dispersal as a major driver, but also indicate that the realized dispersal distances are sometimes difficult to predict based on traits alone (Flinn & Vellend 2005).

Seed sowing experiments – Seed sowing experiments are the most direct way to assess the degree to which dispersal drives plant distributions and abundances (Turnbul et al. 2000; Clark et al. 2007). Although only few seed sowing experiments have been done (Graae et al. 2004; Verheyen & Hermy 2004), the overall result seems to be quite unequivocal: many forest plants are strongly limited by dispersal (but see §3.2 – Recruitment). The importance of seed availability is, however, not only relevant for plant colonization into post-agricultural forest, but also applies to forest plant distributions and abundances in general. A number of studies have introduced seeds into ancient forest patches in which the sown species were absent or already present (Eriksson & Ehrlén 1992; Ehrlén & Eriksson 2000; Ehrlén et al. 2006). As seedlings emerged in both patch types, it was concluded that seed availability plays a key role in structuring forest herb communities.

3.2 Establishment limitation

When diaspores arrive at a post-agricultural forest site, they still need to germinate, recruit into the juvenile and adult life stage, achieve good adult performance and reproduce. If one or more of those successive stages of plant establishment are impeded by the environmental land-use legacies (§2), this will hamper the settlement of a founding population. Yet, research on forest understorey species colonization has focused overwhelmingly on dispersal, while establishment has received much less attention.

Resource alterations – The soil legacies may have either a direct impact on the recruitment and performance of forest plants or an indirect impact via the alteration of interspecific interactions. The increased availability of limiting soil resources (e.g., P; see §2.1) generally leads to increased aboveground biomass production in plant communities, mainly caused by a few rapidly and vigorously growing species (e.g., Al-Mufti et al. 1977; Silvertown et al. 2006; Harpole & Tilman 2007; Hautier et al. 2009). Classical co-existence theory predicts that those species gain competitive dominance by pre-empting resources and exclude other species (Grime 2001; Craine 2005, 2009). Recently, Hautier et al. (2009) showed that the increased aboveground competition for light largely accounts for the exclusion of species growing under the faster-growing or taller species that pre-empt this directionally supplied resource. Within the context of recovering forest plant communities, this means that nutrient enrichment might favour competitive species, which reduce the establishment success or performance of the ancient forest species. *Urtica dioica* L., for instance, is a highly competitive forest herb that strongly responds to increased soil P levels and may form a dense layer (Pigott 1971; De Keersmaeker et al. 2004; Hipps et al. 2005). In alluvial forest, Hermy (1985) for instance found an aboveground biomass for *U. dioica* as high as 1.4 ton. ha^{-1} (± 0.34 SE) in post-agricultural sites compared to 0.26 $ton.ha^{-1}$ (± 0.08 SE) in ancient sites. Despite these detrimental indirect effects, the increased nutrient levels may be, at the same time, beneficial for the growth and performance of the colonizing species. This is a fascinating contradiction that will receive particular attention in the present thesis.

Recruitment – Since dispersal forms a primary limitation on plant colonization, studying the recruitment of species from the seedling into the adult stage mainly relies upon seed sowing experiments. While such studies have been performed for a suite of species (Donohue et al. 2000; Graae et al. 2004; Verheyen & Hermy 2004; Flinn 2007), the species were only monitored for one or two growing seasons. In a meta-analysis of seed sowing experiments, Clark et al. (2007) argued that short-term studies probably underestimate the relative importance of establishment limitation because they do not include additional mortality at the post-seedling stages. To date, the (few) short-term studies do not match the long lifespan of many forest plants (cf. Ehrlén & Lehtilä 2002) and only allow us to determine whether the seedling stage limits plant colonization. While some studies did not find evidence for reduced seedling establishment in post-agricultural sites (Graae et al. 2004; Verheyen & Hermy 2004), others showed that the slow colonization is partly caused by low seedling establishment rates (Donohue et al. 2000; Flinn 2007). Seedling establishment may thus influence colonization, but further long-term monitoring of species introductions is needed to determine whether the subsequent life stages may additionally form bottlenecks for plant colonization. Finally, only Verheyen & Hermy (2004) included a competition treatment in their introduction experiment and showed that seedling emergence and survival were significantly lower when a competing vegetation was present. It is clear that more work on this topic is needed (see §4).

Adult performance – Comparisons of adult plant performance in post-agricultural versus ancient forest have been frequently used to assess the importance of establishment limitation slowing down the colonization of forest understorey species. More specifically, a central question is whether the altered environmental conditions are detrimental or rather beneficial for plant performance. Most studies have measured performance in spontaneously established populations and found equal or greater performance of adult individuals in post-agricultural compared to ancient sites (Donohue et al. 2000; Endels et al. 2004; Fraterrigo et al. 2006; Flinn 2007). Vellend (2005), however, provided clear evidence for the reduced performance of *Trillium grandiflorum* (Michx.) Salisb. in post-agricultural forests. Verheyen & Hermy (2004) used experimentally introduced adult individuals in ancient and post-agricultural stands to determine the effect of former land use on plant performance. Two of the introduced species (*Primula elatior* Hill*, Geum urbanum* L.) showed higher performance in the post-agricultural stands, while the two other species (*Anemone nemorosa* L.*, Ranunculus ficaria* L.) were not affected by the former land use. In sum, these studies show that the former land use altered the environmental conditions in ways that can enhance the performance of some, but not all, forest understorey species (Fraterrigo et al. 2006). An important drawback of the field studies is that they were unable to identify which particular environmental factor accounted for the performance responses to the former land use. Further experimental work on the response of forest plants to one or more environmental factors is thus needed (see §4). An example of such research is the study by Hipps et al. (2005) who showed in a pot experiment that forest herbs may show a morphological (higher biomass) and physiological response (higher P concentrations) to increased P availability. Another shortcoming of most previous field studies is that they mainly looked at morphological responses to the former land use, while other aspects such as seed production and germinability and nutrient uptake received little attention (see §4).

Demographic approach – In their review paper, Flinn & Vellend (2005) argued that the first priority for future work are studies that focus on the demography of particular species, i.e., determine the rates of recruitment into the different life stages, the longevity of individuals and the production of germinable seeds. Only if each establishment stage of a species has been studied, we are able to identify which stage(s) may form a bottleneck for its colonization (Fig. 1.3; §4). To date, only few demographic studies have been done. Donohue et al. (2000) compared the demographic parameters of *Gaultheria procumbens* L. in ancient and adjacent post-agricultural sites and showed that the slow colonization of the species was mainly explained by its slow intrinsic growth rate and low seedling establishment. Using a life table response experiment, Jacquemyn & Brys (2008) modelled the demography of *P. elatior* and found the low seedling and juvenile growth and the low juvenile and adult survival to be the main causes of the slow colonization of the species.

4 Objectives and thesis outline

Research on the impact of land-use change on forest understorey plant distribution and abundance has moved from descriptive studies (e.g., Peterken 1974; Peterken & Game 1984; Wulf 1997; Honnay et al. 1998) to studies focusing on the factors that underlie the persistent impact of former land use on plant populations. The overarching question is *why many forest plant species have such low colonization capacities*. The concise review above expressed the general scientific consensus that low dispersal rates primarily account for the slow colonization into post-agricultural forests. Yet, seeing that former agricultural land use may result in persistent environmental legacies (see §2) and that the impact of those legacies on the different life stages of plant establishment is still poorly understood, further research should focus on the post-dispersal stages of plant colonization.

The main objective of the present thesis is therefore *to identify which plant establishment stage(s) may impede the colonization of forest understorey species into post-agricultural forests*. The focus of the thesis lies on the impact of the fertilization legacy of former agriculture, and P availability in particular. The magnitude and persistence of the P enrichment in post-agricultural forests (see §2.1; Table 1.1) makes the nutrient highly relevant for community development in past and future afforestions. In addition, study sites were mainly alluvial forests because: (1) they are particularly species rich and of high concern for biodiversity conservation (protected with priority in the European Habitat Directive, code 91E0), (2) the cover of the understorey is often dense which implies that aboveground species interactions may be important and (3) an increase in P availability might generate a strong response because most other resources (soil moisture, base cations, nitrogen) are probably not limiting plant growth in rich valley forest soils. Based on the shortcomings of previous studies and the research gaps outlined in §3.2, the specific objectives of this thesis were:

(1) to study *plant recruitment* in introduction experiments that are monitored for more than two growing seasons, i.e., from the seed into the adult life stage, and that include a competition treatment,

(2) to measure the plasticity in *plant performance* in response to particular environmental conditions (here mainly P availability) in both field and experimental studies,

(3) to study recruitment and performance for the same suite of species, i.e., seeking for maximal integration and complete coverage of the different establishment stages for those species,

(4) to determine the long-term spontaneous community development in post-agricultural versus ancient forests, i.e., to look at the actual colonization under field conditions (integration of all life stages).

The first phase of plant establishment is the recruitment of seeds into the seedling, juvenile and adult life stage (Fig. 1.3; Fig. 1.4). **Chapter 2** describes an introduction experiment in which ten species were introduced in two contrasting forest types (valley, plateau) with or without the removal of the competing vegetation. Recruitment into the adult life stage was monitored for five growing seasons. **Chapter 3** contains a similar experiment that zooms in on two species (*Primula elatior, Geum urbanum*), which were monitored in greater detail for eight growing seasons.

Achieving good adult performance forms the second phase of plant establishment. In a field study, we determined the uptake of nutrients (N and P) and biomass production of seven forest herbs in post-agricultural versus ancient forest sites (**Chapter 4**). Plant material was collected in the introduction experiments of Chapters 2 and 3. Next, we performed a pot experiment to explicitly test the plasticity of four forest herbs (P uptake, biomass and other traits) in response to P and light availability (**Chapter 5**). **Chapter 6** looks at the role of P availability in the production of germinable seeds in ancient and post-agricultural forest and determines the role of P. Seed were collected from the plant individuals studied in Chapters 3 and 5.

Finally, **Chapter 7** describes how forest plant communities changed during 30 years of spontaneous forest development in ancient and post-agricultural valley forest. If dispersal limitation is the only constraint for plant colonization, we expect forest plants to gradually colonize the post-agricultural sites so that ancient and post-agricultural understorey communities become more similar over time. The validity of this hypothesis will be evaluated here. The results of the experiments in Chapters 2–6 and the spontaneous community development over three decades are summarized in **Chapter 8**. This final chapter additionally presents a conceptual model of community development in post-agricultural forests.

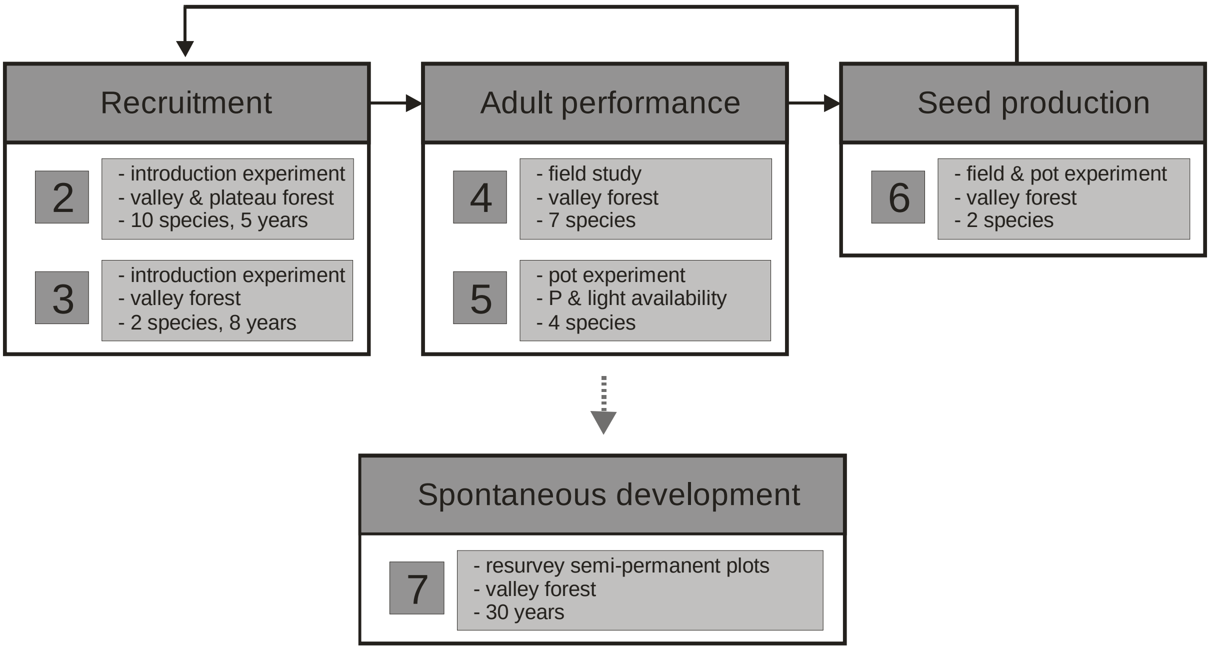


Fig. 1.4 Outline of the thesis indicating the four main parts: recruitment, adult performance, seed production and 30 years of spontaneous understorey community development. The chapter numbers are indicated by the numbers 2–7. The results from the first three parts (Chapters 2–6) will be useful to explain the long-term community development (Chapter 7).

CHAPTER 2 – LOW RECRUITMENT ACROSS LIFE STAGES PARTLY ACCOUNTS FOR THE SLOW COLONIZATION OF FOREST UNDERSTOREY PLANTS

AFTER: BAETEN L, JACQUEMYN H, VAN CALSTER H, VAN BEEK E, DEVLAEMINCK R, VERHEYEN K, HERMY M (2009) LOW RECRUITMENT ACROSS LIFE STAGES PARTLY ACCOUNTS FOR THE SLOW COLONIZATION OF FOREST HERBS. *JOURNAL OF ECOLOGY* 97, 109-117.

Abstract

Former land use has long-lasting effects on the distribution and abundance of forest understorey plant species. Previous studies mainly focussed on limited dispersal capacities of forest plants 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. We examined recruitment of ten forest plants 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. 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 benefitted from clearing; vernal geophytes were not affected. Post-seedling mortality further reduced recruitment success into the adult life stage. Because only a fraction of seeds effectively recruited into the adult life stage, forest plants need high seed densities for colonization. Seed availability is, however, severely limited by the low dispersal capacity of many forest plants. Disturbed microsites promoted recruitment of some, but not all forest plants. The effect was most clear in valley forest, probably because the established vegetation had high cover and was dominated by competitive herbs. 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.

1 Introduction

It is well known that human disturbances have long-lasting effects on the 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; Chapter 1). Besides the obvious consequences for conservation, sites recovering from disturbance provide ideal settings for assessing key drivers of community assembly because species need to colonize unoccupied habitat patches. More generally, it is questioned to which extent species distributions and abundances are explained by dispersal or by environmentally 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 the forest understorey diversity in these post-agricultural forests remains impoverished for decades to centuries because many forest plant species have a low colonization capacity (reviewed in Flinn & Vellend 2005; Hermy & Verheyen 2007). From the review in Chapter 1 it is clear that an overwhelming amount of studies have demonstrated 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 their colonization capacities, i.e., the slowest fern species had the lowest recruitment.

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; Chapter 3). Third, the extent of recruitment limitation of many forest plants 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 the present chapter is the evaluation of recruitment limitation of forest understorey plant species according to these issues. We present results of a seed sowing experiment including ten forest plants 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 two 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.

2 Methods

2.1 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: (1) 'valley' forest on rich, moist soils and (2) 'plateau' forest on less rich, drier soils. Each forest type was represented by two recent forest stands within the study region, i.e., 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 plants within this area and the 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 (± standard error, mg/100 g dry soil) based on eight mixed 0–10 cm soil samples were 389.0 ± 20.0 total nitrogen (total N, Kjeldahl method) and 15.4 ± 1.8 K^+, 23.0 ± 2.1 Mg^{2+} and 271.2 ± 40.4 Ca^{2+} (ammonium-acetate EDTA extraction, Van Ranst et al. 1999). Average phosphorus (P) concentration (ammonium-acetate EDTA) was 2.1 ± 0.3 mg/100 g dry soil, which is three times higher than the typical P levels in forest without agricultural history (*c.* 0.75 mg/100 g, Chapter 3). 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. (1999b) present detailed results on plant 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 Mg^{2+} and 21.7 ± 3.6 Ca^{2+} (± standard error, 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).

Differences in productivity between valley and plateau forest were reflected in the 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 % (± 2 % SE) and in plateau forest only 34 % (± 4 % SE). A second critical difference between both forest types was the dominance of the competitive herb *Urtica dioica* L. (cf. De Keersmaeker et al. 2004), which covered on average 35 % (± 4 % SE) in valley forest, whereas this herb was absent from plateau forest.

Ten characteristic forest plants, six in each forest type, were introduced as seeds. Two species, *Hyacinthoides non-scripta* (L.) Chouard 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 2.1. All, except for *Geum urbanum* L., have proven to be slowly colonizing forest species (Verheyen et al. 2003c), which are generally confined to ancient forest sites. Species with different life forms (geophytes, hemicryptophytes and chamaephytes) and leaf phenology were selected to exclude bias in our results towards certain species groups.

Table 2.1 Forest understorey species 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
Anemone nemorosa	valley & plateau	200, 1000, 4000	herb	geophyte	vernal
Hyacinthoides non-scripta	valley & plateau	80, 400, 1600	herb	geophyte	vernal
Carex sylvatica	valley	200, 1000, 4000	graminoïd	hemicryptophyte	evergreen
Geum urbanum	valley	200, 1000, 4000	herb	hemicryptophyte	evergreen
Paris quadrifolia	valley	36, 180, 720	herb	geophyte	aestival
Primula elatior	valley	200, 800, 1600	herb	hemicryptophyte	evergreen
Lamium galeobdolon	plateau	60, 300, 1200	herb	chamaephyte	evergreen
Milium effusum	plateau	200, 1000, 4000	graminoïd	hemicryptophyte	evergreen
Oxalis acetosella	plateau	40, 200, 800	herb	chamaephyte	evergreen
Polygonatum multiflorum	plateau	28, 140, 560	herb	geophyte	aestival

2.2 Design and monitoring

The experiment was initiated during 2002. The design corresponds to a factorial experiment with the treatments fully crossed: (1) the vegetation and litter was cleared or left intact (‘cleared’ and ‘undisturbed’) and (2) seeds were sown in three species-specific densities (Table 2.1). In half of the experimental 1.75 m × 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 × 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 species were sown in separate 0.5 m × 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* 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 (see Chapter 3 for more details on the species' ecology).

2.3 Data analysis

The data were analysed in two successive steps: (1) germination success two years after seed sowing and (2) ultimate recruitment of adult individuals after five growing seasons. Further, the analysis of both steps was performed at the subplot (individual species) and the plot level (experimental community). '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 two years (2004) in each plot were tested using permutational analysis of variance (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 two years (2004) and five years (2007) after seed sowing were tested with a non-parametric Wilcoxon Signed-Rank test. 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, i.e. post-seedling mortality, was related to initial seed density by means of bivariate Spearman Rank correlations.

The count data of subplots, containing the six different forest plants, were combined for every plot. This plot level can be viewed as an experimental community in which forest plants 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 (ter Braak & Šmilauer 2002; 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 non-parametric Kruskall-Wallis tests. To test for effects of clearing and seed density on overall recruitment at the plot level, we ran a permutational MANOVA in PERMANOVA (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).

3 Results

3.1 Germination at the species and community level

In the moist valley forest type, the sown species (except for the two vernal geophytes *H. non-scripta* and *A. nemorosa*) showed significantly higher germination in cleared plots (Table 2.2). 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* All. whereas higher seed densities resulted in more recruits in four out of six species.

Table 2.2 Effect of vegetation and litter clearing and seed density on the number of recruits of each species two years after seed sowing (n = 48 per species).

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

Notes: 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 seed densities.

Results at the level of the experimental community, combining all species at the plot level, were largely analogous (Table 2.3a). 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 the composition at the plot level. Cleared and undisturbed plots were significantly separated along the second PCA axis (Fig. 2.1; $\chi^2 = 23.27$, $P < 0.001$) whereas seed density did not significantly separate along the first and second axis. In the plateau forest type, only seed density had a (highly) significant effect on the number of recruits per plot across species (Table 2.3a). Species abundances were all positively intercorrelated (Fig. 2.1) and seed densities separated significantly along the first PCA axis ($\chi^2 = 31.90$, $P < 0.001$).

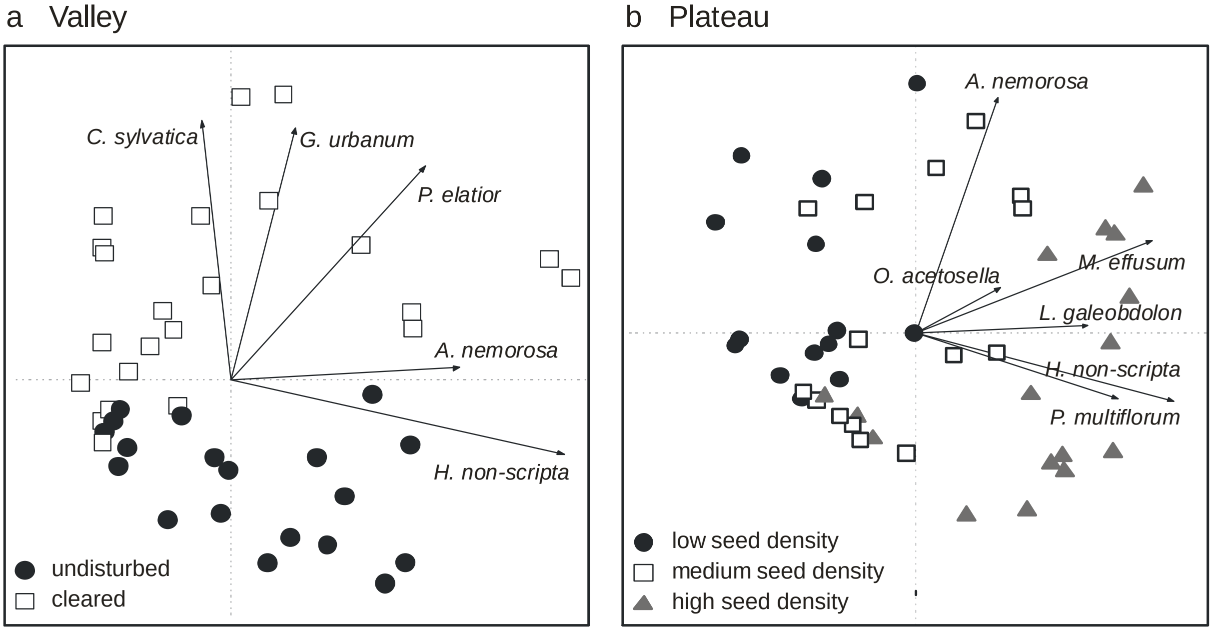


Fig. 2.1 Correlation bi-plot of a PCA analysis on the number of recruits of the sown species in each plot two years after seed sowing. The first two ordination axes account for 69.3 % (a) and 70.1 % (b) of the total variance.

3.2 Recruitment into the adult life stage: change 2004–2007

In the cleared plots of the valley forest stands, the per-seed-response of the two hemicryptophytes *G. urbanum* and *P. elatior* significantly decreased between 2004 and 2007 (Fig. 2.2c). Thus, for these forest plants, the number of recruits per seed two years after seed sowing was higher than the number of recruits per seed that remained after five years. In undisturbed plots, the initial low per-seed-response of these two 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 (Fig. 2.2b,d). 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, i.e., the proportion of individuals that did not survive to the adult stage (2007), was lower at higher seed densities.

Because the recruitment success of the hemicryptophytes in the cleared plots in valley forest decreased between 2004 and 2007, the clearing treatment did no longer significantly affect composition at the plot level after five growing seasons (Table 2.3b). 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.

Table 2.3 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 (n = 48).

Source of variation	Valley forest		Plateau forest	
a) Germination (2004 data)				
Clearing	3.68	*	0.47	NS
Seed density	4.04	***	42.11	***
Clearing × 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 × seed density	0.16	NS	0.06	NS

Notes: Test statistic: pseudo-F value based on a permutational analysis of variance (Anderson 2001); significance tested through 999 randomizations (^{NS}: not significant; *: $P \leq 0.05$; ***: $P \leq 0.001$).

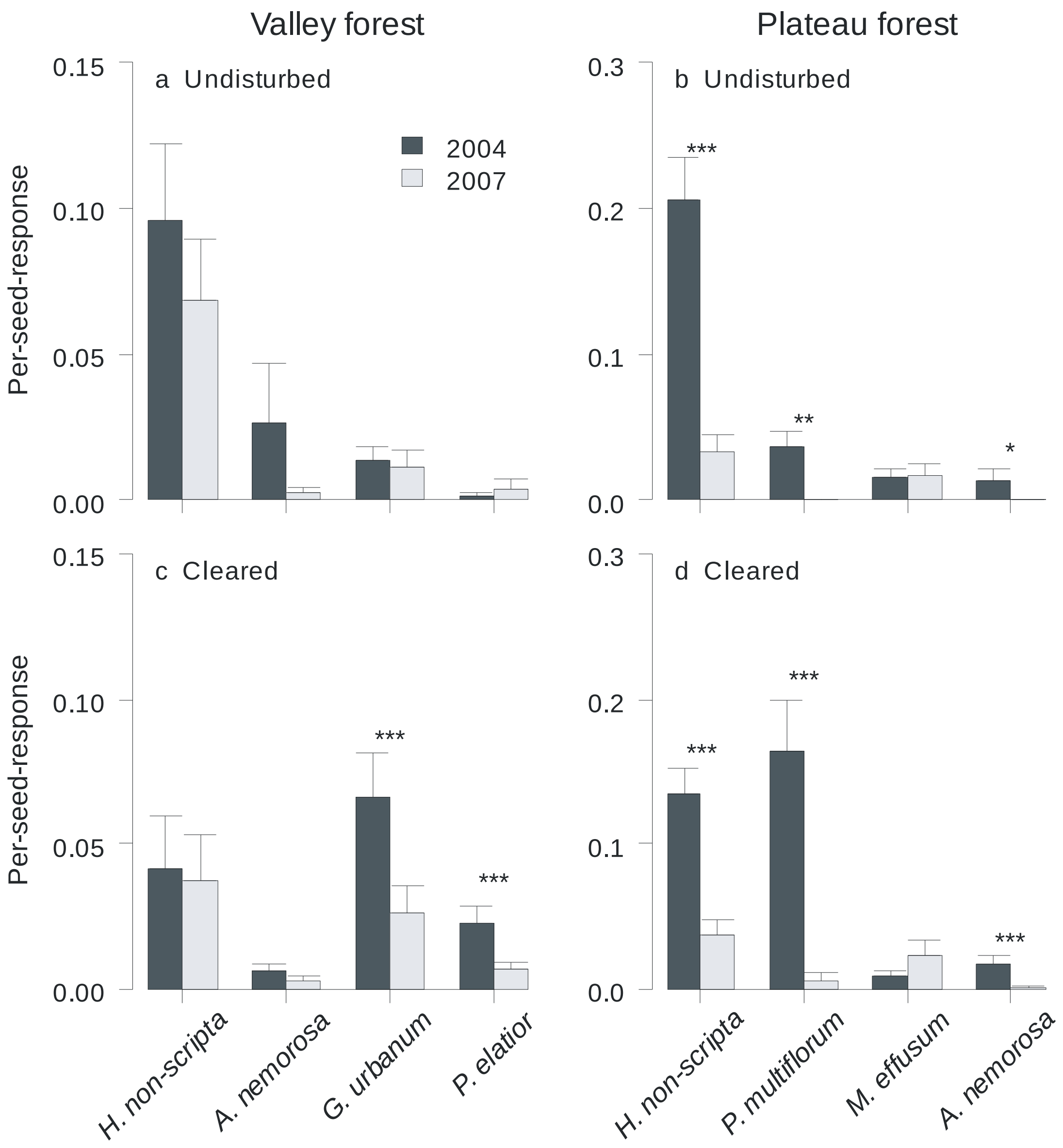


Fig. 2.2 Mean number of individuals per subplot in 2004 and 2007 standardized by the number of seeds added (per-seed-response). Significant differences between years (Wilcoxon Signed-Rank) are given above bars; *: *P* ≤ 0.05; **: 0.001 < *P* ≤ 0.01; ***: *P* ≤ 0.001. Error bars represent standard errors (n = 48 for each species in each forest type).

In undisturbed plots, the number of individuals across the three life stages of *G. urbanum* and *P. elatior* was low (Fig. 2.3a,b). Only a low number of seedlings and vegetative individuals recruited two years after seed sowing (2004) so that further growth to a reproductive stage (2007) was very limited. In cleared plots, recruitment two years after seed sowing (2004) of both *G. urbanum* and *P. elatior* was more successful (cf. Table 2.2). 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.

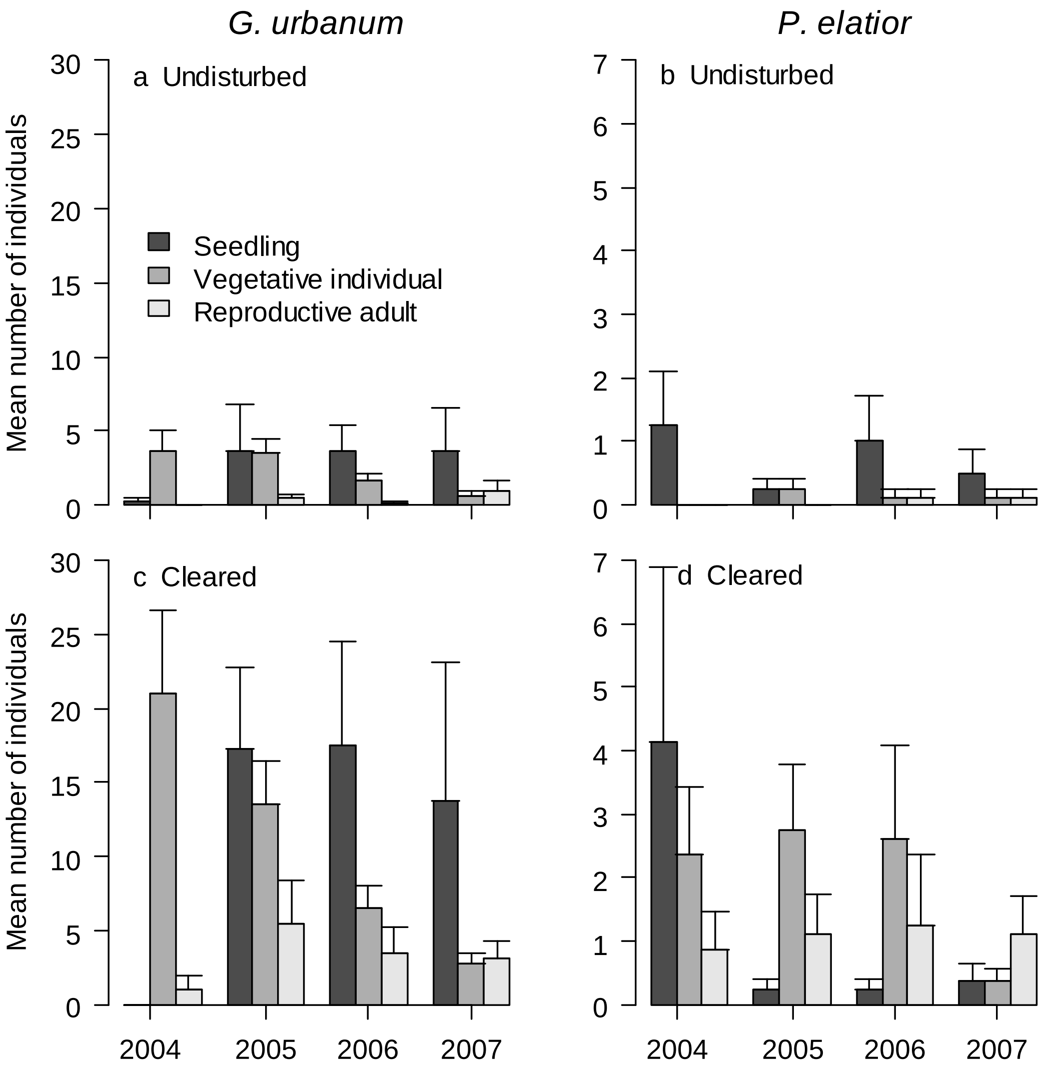


Fig. 2.3 Distribution of all individuals per subplot of *G. urbanum* (4000 seeds.m^{-2}) and *P. elatior* (1600 seeds.m^{-2}) across tree life stages between 2004 and 2007. Error bars represent standard errors (n = 16 per species).

4 Discussion

The experimental results presented in this study clearly indicate that dispersal constraints can severely limit the colonization of forest understorey plant species. Higher seed densities resulted in more recruits in eight out of ten species and positively affected abundances at the plot level. The positive effect of seed availability on species abundances persisted at least for five years following seed sowing. Our results thus agree with the extensive work on recovering forests confirming the major importance of dispersal limitation to explain distributional patterns of forest plant species (e.g., Matlack 1994; Bossuyt et al. 1999b; Verheyen & Hermy 2001a,b; Chapter 1). 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 plants. 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.

4.1 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). The role of seed quantity and quality forms the subject of Chapter 6.

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. 2.1). Second, a vegetation survey in all plots showed that the competitive herb *U. dioica* dominated our experimental plots (average cover 35 % ± 4 % SE). This species is known to profit from high P availability, a persistent legacy from former agricultural land use, and can hamper the colonization of forest plants (e.g., Hermy et al. 1999; Honnay et al. 1999; De Keersmaeker et al. 2004; Hipps et al. 2005). P is therefore an indirect, via *U. dioica*, hindrance to community recovery. Slow colonization of forest plants 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 the 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 (Fig. 2.4). 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 plants and also found no evidence of competition for light between forest plants 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. 2.1). 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.

Fig. 2.4 Example of an introduction site in plateau forest showing the presence of a thick litter layer.

4.2 Recruitment to the adult life stage

The number of adults that recruited five years after seed sowing was generally lower than the number of recruits observed after two 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. Chapter 3 reports such a detailed demographic study for *P. elatior* and *G. urbanum* during eight growing seasons after their introduction in both ancient and post-agricultural forest.

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. 2.2) 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 plant species to disperse (e.g., Graae & Sunde 2000; 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. 2002b).

It has been suggested in other seed sowing experiments that certain forest plants 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. 2.2). Therefore, only five years after microsite creation, abundances of the sown species did no longer differ between cleared and undisturbed plots (Table 2.3). 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 five 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. 2.3). 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. Chapter 3 will further elaborate on the different demography of *P. elatior* and *G. urbanum*.

In conclusion, we showed that dispersal limitation alone is insufficient to account for the low colonization rates of forest understorey species during forest recovery. Rather, limited dispersal coupled with a small fraction of seeds that effectively recruits into the adult life stage explains plant colonization. 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.

CHAPTER 3 – ESTABLISHMENT LIMITATION CONTRIBUTES TO THE DIFFERENTIAL COLONIZATION CAPACITY OF *PRIMULA ELATIOR* AND *GEUM URBANUM*

AFTER: BAETEN L, HERMY M, VERHEYEN K (2009) ENVIRONMENTAL LIMITATION CONTRIBUTES TO THE DIFFERENTIAL COLONIZATION CAPACITY OF TWO FOREST HERBS. *JOURNAL OF VEGETATION SCIENCE* 20, 209-223.

Abstract

The recovery of forest plant communities in post-agricultural landscapes is largely determined by dispersal constraints, but can environmental legacies of former land use additionally limit the colonization of post-agricultural forest by forest understorey species? Seeds and adults of two forest herbs with similar life-history traits, but contrasting colonization capacity – the fast colonizing *Geum urbanum* and the slow colonizing *Primula elatior* – were introduced in both ancient and post-agricultural forest sites. Soil conditions and plant tissue nutrient concentrations were measured to characterize habitat quality. To determine whether the introduced species could successfully establish and persist we monitored recruitment, longevity and adult performance during eight years in permanently marked plots. The results showed that P availability was ten times higher in post-agricultural forest soils and was also reflected in the plant tissue samples. Species longevity was clearly lower in post-agricultural forest sites indicating higher turnover. The fast colonizing *G. urbanum* counterbalanced this lower longevity by new establishment, the slow colonizing *P. elatior* dropped below the number of originally introduced individuals. Additionally, *G. urbanum* performed better in post-agricultural forest sites contrary to *P. elatior*. Thus, even when dispersal constraints of the slow colonizing forest herb *P. elatior* are eliminated through introduction, environmental conditions in post-agricultural forest sites additionally restrict its recruitment, longevity and performance. These experimental results suggest that environmental constraints may strengthen the differences in colonization capacity among forest plants if slow dispersers also tend to be less likely to establish.

1 Introduction

Across many landscapes throughout Europe and eastern North-America considerable areas of forest are recovering from large scale land-use changes such as the past clearance for agricultural purposes (e.g., Foster et al. 1998; Kirby & Watkins 1998; Flinn & Vellend 2005). Numerous studies report long-term reduced forest understorey plant diversity in post-agricultural versus ancient forests because many forest understorey plant species (i.e., ancient forest species *sensu* Hermy et al. 1999) have an extremely low ability to colonize the post-agricultural forests (see Hermy et al. 1999; Flinn & Vellend 2005; Hermy & Verheyen 2007 and Chapter 1 for a review). The observation that forest species may greatly differ in their capacity to colonize post-agricultural forests (e.g., Verheyen et al. 2003c) raises questions about which mechanisms underlie the colonization process. Addressing these questions is not only of interest to restoration ecologists, but will also reveal fundamental processes driving community assembly in these forests.

The failure of particular forest understorey plants to colonize post-agricultural forests is generally placed within a dispersal limitation versus establishment limitation framework in which either species dispersal or local abiotic and biotic interactions govern community assembly. A vast amount of studies have focused on the dispersal stage of plant colonization and, to date, it is generally accepted that the differential dispersal capacity of forest plants governs the community assembly in post-agricultural forests (e.g., Matlack 1994; Brunet & von Oheimb 1998; Verheyen et al. 2003c; Flinn & Vellend 2005; Vellend et al. 2007; Chapter 1 §3.1). However, since the former land use has often persistently altered the environmental conditions (e.g., Koerner et al. 1997; Verheyen et al. 1999; Dambrine et al. 2007; Chapter 1 §2), it seems likely that altered habitat quality imposes additional constraints on the establishment of slow colonizing forest plants suggesting, at least partial, establishment limitation (Honnay et al. 1999; Vellend 2005; Flinn 2007; Chapter 2).

Experimental introductions of fast and slow colonizing forest species are probably the most straightforward way to determine whether a differential establishment success contributes to their contrasting colonization rates. Yet, only few studies have performed such experiment. Graae et al. (2004) and Verheyen & Hermy (2004) sowed seeds of fast and slow colonizers in ancient and post-agricultural sites and monitored seedling emergence for two growing seasons. The initial recruitment of both the fast and the slow colonizing species was equal or higher at the post-agricultural sites, so germination and seedling establishment seemed not to be the critical phases. On the other hand, Flinn (2007) found consistently higher initial recruitment rates for a fast colonizing compared to a slow colonizing fern species and suggested that the limited recruitment controlled colonization. Since the relative importance of establishment limitation versus dispersal limitation is expected to increase when additional mortality during subsequent life stages is included (Clark et al. 2007; Chapter 2), detecting an effect of habitat quality differences between ancient and post-agricultural forest on the colonization of forest plants may require long-term monitoring. Looking at the colonization of post-agricultural forests as a bottleneck process (Fig. 1.3), establishment limitation can result from only one life stage that is constrained by habitat quality, regardless performance in other stages including successful germination.

In the present chapter, we present the establishment success of two experimentally introduced forest herb species through multiple life stages, following up on the initial germination results reported in Verheyen & Hermy (2004). *Primula elatior* Hill and *Geum urbanum* L. are two understorey forest herbs with similar life-history characteristics, but contrasting colonization capacity. The species were introduced in ancient and post-agricultural forest stands by means of seed sowing or planting adults in undisturbed plots and in plots with the aboveground vegetation cleared. Our aim was to test for environmental constraints on several demographic stages that may account for differences in the colonization capacity between the two forest herbs. Just like in Chapter 2, we determined recruitment into the adult stage (here for eight growing seasons), but extended this monitoring by additionally measuring longevity and adult performance. Though the main focus is on *P. elatior* and *G. urbanum*, qualitative observations on a similar pair of forest herbs with contrasting colonization capacities (*Anemone nemorosa* L. and *Ranunculus ficaria* L.) are also discussed. We hypothesized that: (i) there are persistent differences between ancient and post-agricultural forest patches in (a)biotic conditions, which make establishment limitation likely; (ii) recruitment success, longevity and performance of the introduced forest herbs differ significantly between ancient and post-agricultural forest sites; (iii) this differential recruitment, longevity and performance between ancient and post-agricultural forest sites corresponds to the different colonization capacity of the studied forest herbs.

2 Methods

2.1 Study site and studied species

The study was conducted in the 34 ha forest Muizenbos located in a flat area (10 m height above sea level) in northern Belgium. Soils are Quaternary niveo-eolian deposits varying from silty sand to sandy silt (see Verheyen et al. 2001 for details on soil properties) harbouring a natural forest plant community of rich and mesic sites. The northern, acid part of the forest belongs to the *Violo-Quercetum roboris* whereas the more calcareous central part belongs to the *Primulo-Fraxinetum excelsioris* (Hermy 1985) with planted poplar cultivars (*Populus* × *canadensis*) dominating the tree layer. The entire forest consists of forest stands with different land-use histories studied in detail in Verheyen & Hermy (2001a,b). This mosaic of stands with different ages results from parcels that were cleared for agricultural use during the nineteenth and twentieth century and subsequently afforested in different phases combined with stands that have probably been continuously forested (Fig. 1.1). These different land-use histories are reflected in the soil properties, especially phosphorus (P) availability (De Keersmaeker et al. 2004), and species distributions (Verheyen & Hermy 2001a,b) of the present day forest.

In this study, we focus on two species with similar life histories, but contrasting colonization capacity. A short introduction to the species' ecology is give here and includes their seed and dispersal characteristics, which are also relevant for Chapter 6. *Primula elatior* (oxlip) is a small, perennial rosette hemicryptophyte of deciduous temperate forests. The species is widely distributed in western and central Europe; it occurs from Denmark in the north to the Pyrenees in the south and extends its range eastwards to central Asia (Taylor & Woodell 2008). *Primula elatior* is typically associated with late-successional 'ancient' forest (Hermy et al. 1999; Verheyen et al. 2003c), but occasional colonization of post-agricultural forest has been observed as well (e.g., Jacquemyn et al. 2002). Its vegetative spread via short rhizomes is very limited, so seed dispersal primarily drives the colonization of *P. elatior*. The species is distylous and selfincompatible and produces on average 29.18 (± 26.30 SD) fruits per plant in late May–early June; the number of seeds per fruit may vary considerably (mean 34.72 ± 17.29 SD) (Jacquemyn et al. 2009). Seeds of *P. elatior* lack adaptations for specific dispersal mechanisms, so seeds are usually dispersed around the mother plants through barochory. However, occasional long-distance dispersal may occur, e.g., by deer and pheasants that foraged on the fruits. Seeds germinate in spring after their dormancy has been broken by a cold period (Taylor & Woodell 2008). An extensive review of the species' distribution and autecology can be found in Taylor & Woodell (2008).

Geum urbanum (herb bennet) is also a small, perennial rozette hemicryptophyte of deciduous temperate forests. The distributional range of the species is wider than that of *P. elatior* and basically covers the whole of Europe except for the most northern parts of Scandinavia and Iceland. Outside Europe, it occurs eastwards to western Asia, western Siberia and the Himalaya and is common on the northwest African coast (Taylor 1997). *Geum urbanum* is not confined to ancient forest and is considered a good colonizer of post-agricultural stands (e.g., Verheyen et al. 2003c). The vegetative spread via short rhizomes is limited, so it mainly reproduces by seeds. The species is selfcompatible and forms flowering heads with *c.* 100 achenes and often more than 1000 seeds per plant from July to September. The achene (further referred to as 'seed') is the dispersal unit for the species and has a strongly hooked awn, which enables seeds to attach to animal furs (epizoochory). Germination can occur immediately after dispersal (e.g., Verheyen & Hermy 2004), but mainly occurs in the spring following seed set, with a peak in March. Seeds do not need a cold stratification to germinate. An extensive review of the species' distribution and autecology can be found in Taylor (1997).

In the original Verheyen & Hermy (2004) study, two additional forest herbs with a different growth form (geophytes) were introduced: the slow colonizing *A. nemorosa* and the fast colonizing *R. ficaria* (cf. Verheyen et al. 2003c). Both species were monitored in detail during the first two growing seasons (Verheyen & Hermy 2004), but afterwards only qualitative observations (frequency of occurrence and percentage cover per plot) were made because of their strong vegetative growth, which complicated the distinction of new recruits via seed versus vegetative growth of adults. Therefore, these two species were not analysed in detail, though the qualitative observations will be presented in the discussion section.

2.2 Experimental design and characterization of the (a)biotic environment

The experimental design was very similar to that used in Chapter 2. To study recruitment, longevity and adult performance of the two forest species in forest environments with contrasting land-use history, four post-agricultural (*c.* 15 year) forest sites on former heavily fertilized grassland and two sites under at least > 145 years (oldest available land-use map) continuous forest cover were selected. Both ancient and post-agricultural sites were carefully chosen to have a similar tree layer (poplar cultivars planted in wide spacings) and sub-canopy species composition (*Alnus glutinosa* (L.) Gaertn., *Fraxinus excelsior* L.) resulting in similar light conditions. The experiment was designed as a generalized randomized block design (*sensu* Neter et al. 1996), i.e., the treatments were replicated within blocks (here: forest sites). In every forest site, twelve 2 m × 2 m plots were regularly distributed in a *c.* 12 m × 12 m homogeneous, fenced area and were permanently marked (Fig. 3.1). Each plot comprised four subplots and each of the four studied forest herbs was randomly introduced in one such subplot in 1999. In half of the plots, the species were introduced as seeds in June 1999. Seed densities were 400 seeds.m^{-2}. In the remaining plots, adult individuals of *P. elatior* (four per subplot) and *G. urbanum* (two per subplot) were planted immediately after collection in April 1999. In addition, the aboveground biomass of the established vegetation was cleared in half of the seeded and half of the planted plots during the first three years following the introduction (1999–2001) to allow initial competition free growth. Plots with continuous cover of the established vegetation will be further referred to as 'undisturbed' plots, whereas those with initial vegetation removal as 'cleared' plots. Supplementary information on the experimental design and exact location of the forest sites can be found in Verheyen & Hermy (2004).

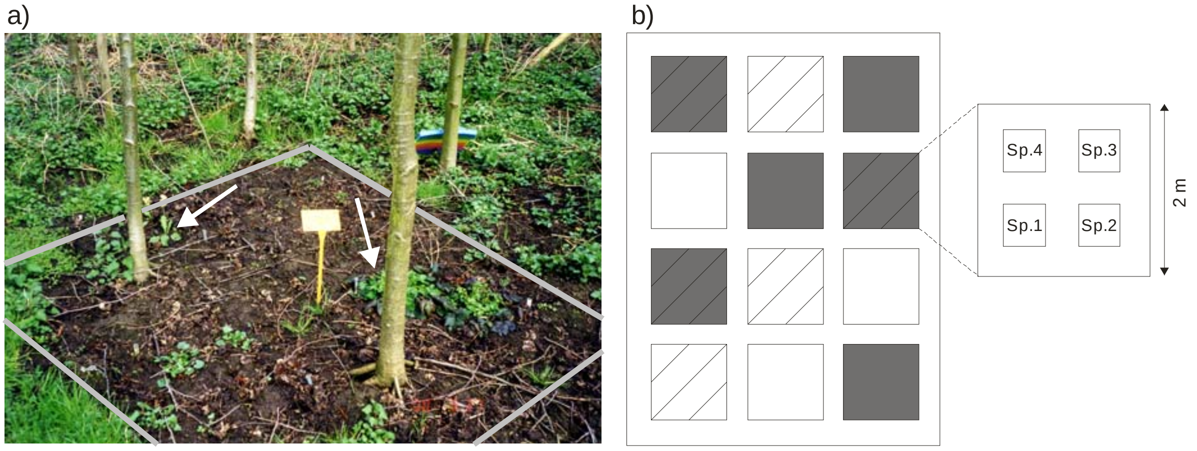


Fig. 3.1 (a) Example of an experimental plot in which the aboveground vegetation was cleared. White arrows indicate planted individuals of *P. elatior* and *G. urbanum* (photograph Kris Verheyen). (b) Design of one experimental block, i.e., forest site. In half of the 2 m × 2 m plots the aboveground vegetation was cleared (hatched plots). The four species were introduced into the four subplots (Sp. 1–4) as seeds (grey plots) or adults (white plots).

Analogous to Verheyen & Hermy (2004), we took five soil samples of the topsoil layer (0–10 cm) at each site in May 2007 to characterize the abiotic soil properties that are likely to be affected by agricultural land use. These soil samples were dried for 48 h at 40 °C to constant weight and subsequently combined into a mixed soil sample per site. The soil samples (< 2 mm fraction) were analysed for pH (pH-KCl), carbon content (loss on ignition at 450 °C) and ammoniumacetate-EDTA extractable P, K^+, Mg^{2+} and Ca^{2+} (Van Ranst et al. 1999). Additionally, five large flowering adults of both introduced species (*G. urbanum* and *P. elatior*) were collected from each site in May 2007. Roots and shoots of these samples were separated and dried for 48 h at 70 °C to constant weight. Then, all five biomass samples per site were combined into a mixed root or shoot sample proportionally to their dry weight. These mixed samples were analysed for total nitrogen (N) (Kjeldahl-method), total P and total potassium (K) (both $HClO_4$ destruction) content. Overall differences in P, N and K tissue concentration between post-agricultural and ancient forest sites were tested with a Mann-Whitney test on pooled root and shoot data of both species. Tissue samples were only taken in 2007 because P, N and K availability was not expected to have changed significantly during the experimental period due to the persistence (P), active biogeochemical cycling (N) and presence of calcareous bedrock (K). Concentrations of K^+ (soil and plant), Mg^{2+} and Ca^{2+} in the extracts were analysed with atomic absorption spectroscopy (Van Ranst et al. 1999), concentrations of P with the colorimetric method of Scheel (1936).

To account for interactions between the introduced species and the surrounding vegetation, especially the competitive herb *Urtica dioica* L. (cf. Honnay et al. 1999; De Keersmaeker et al. 2004), the vegetation composition of every plot was characterized with a vegetation survey in April 2007. In each 2 m × 2 m plot, the occurrence of all understorey species, including the introduced species, and their estimated percentage cover was recorded. Detrended correspondence analysis (DCA) in CANOCO 4.5 (ter Braak & Šmilauer 2002) was used to visualise differences in vegetation composition between plots. Differences in total sum of cover of herb layer species and differences in cover of *U. dioica* were tested with a Mann-Whitney test with forest age as grouping variable.

2.3 Monitoring procedure and data analysis

The experiment was monitored in a hierarchical way from forest site to individual traits resulting in data of increasing detail. The abiotic environment was characterized for every forest site separately (see above) because soil properties are assumed to be strongly affected by the land-use history, which varies at this level. Recruitment of new individuals was monitored at the plot level in both the plots where the species were introduced as seeds and adults. For the determination of longevity, we further zoomed in on the subplot level. Here, only subplots with transplanted adults were used because only these individuals could be accurately relocated each year. Finally, adult performance of the introduced species was assessed at the level of individual plants (traits). Combining these data results in a complete coverage of the different demographic stages, which leads to an integrated approach of establishment limitation and extends the experimental results from Chapter 2 (cf. Flinn & Vellend 2005).

Recruitment of adult individuals was recorded in all 72 plots, i.e., in plots where species were introduced as seeds and in plots where species were introduced as adults. Hereto, the total number of individuals of the studied species was counted in each of the four subplots within that plot between 2000 and 2007, except for 2002 and 2003. A distinction was made between seedlings, non-flowering and flowering adult individuals. Inspecting each of the four subplots, instead of only the subplots where the species under consideration was introduced, was mainly done for two reasons. First, the monitoring of seedling emergence in the other three subplots allowed correction for natural seedling dispersion into the experimental plots (Reader 1993; Verheyen & Hermy 2004). Only for *R. ficaria*, not discussed here, spontaneous seedlings were observed from the first year on. Second, the small 0.5 m × 0.5 m dimension of the subplots was initially necessary to make accurate counting of seedlings feasible, but was not large enough to contain more than a few adult individuals and would ignore expansion of the introduced species. We acknowledge that it is not completely certain that seedlings or adults in the three other subplots originate from the introduced individuals in the adjacent subplot. Nevertheless, because seedling emergence was only observed after seed setting of the introduced individuals, we argue that these individuals most likely originate from the introduced propagules. The effects of forest age and vegetation clearing on the total number of adult individuals were tested with Generalized Linear Mixed Models (GLMMs) with poisson errors for count data and log link function (Zuur et al. 2009). Because forest age varies at the site level (two ancient, four post-agricultural sites), we included 'Site' as a random effects term in the model. The models were fitted with the *lmer* function in the *lme4* package in R 2.9.1 (R Development Core Team 2009; Bates & Maechler 2010).

The longevity of the adults was assessed by counting the number of surviving original transplants yearly (except for 2002–2003) in the permanently marked subplots with planted individuals. Significant effects of forest age and vegetation clearing on the proportion of surviving individuals over the entire monitoring period were tested with GLMMs. As the survival was calculated as the proportion of living individuals versus the total number of introduced individuals in 1999, i.e., proportional data, we used binomial errors and logit link function. Again, ‘Site’ was included as random effects term. To account for the repeated measurements on the same subplots (2000–2007), we also included ‘Subplot’ in the model as random effects term (Zuur et al. 2009). Thus, ‘Site’ and ‘Subplot’ were non-nested random effects terms. Interactions were only included if significant. When combining the survival data with the recruitment data a distinction could be made between the number of original transplanted and newly established individuals. This was not possible for seeded plots because the exact location of the individuals that originated from the 1999 seeds was not known.

Measurements of five life history traits related to growth and reproduction were used to characterize the performance of the adult individuals (cf. Endels et al. 2004; Vellend 2005). Two vegetative traits (number of green leaves per plant, length of the longest leaf) and three generative traits (number of inflorescences per plant, length of the longest inflorescence, number of flower buds/flowers) were measured on a maximum of four (*P. elatior*) and two (*G. urbanum*) adult individuals per plot. Analogous trait measurements were made in 2001 (Verheyen & Hermy 2004) and in 2007. In 2001, the trait measurements were made on the transplanted individuals since they generally were the only individuals present. Due to the mortality of many adult individuals of the 2001 survey, an adjusted selection procedure of adults was adopted in 2007, that is, trait values were derived from the largest, flowering adults, regardless of origin. The five plant traits were combined into a single synthetic plant performance index (PPI) for each species and year separately (because of the different selection procedure) by means of principal component analysis. For each species and each year the first component accounted for more than 50 % of variance of the trait data and was positively correlated with the trait values (see Appendix 3.2). This component was therefore used as an index of increasing performance. The effects of forest age and vegetation clearing on the PPI were tested with GLMMs models with ‘Site’ as a random effects term and normal errors.

3 Results

3.1 Characterization of experimental sites

The different land use history of the forest stands was mainly reflected in the plant-available P content of the upper soil layer (Table 3.1). The post-agricultural forest sites contained up to ten times more P than the ancient forest stands. This difference was also found in the plant tissue nutrient concentrations of *G. urbanum* and *P. elatior* (Fig. 3.2). A Mann-Whitney test on pooled data with forest age as a grouping factor indicated that total P content in roots and shoots was significantly higher in post-agricultural forest compared to ancient forest (Z = -4.799, $P < 0.001$). Total N and K concentrations, on the other hand, were mainly determined by plant species (for N) and part of the plant (for N and K) and did not significantly differ between ancient and post-agricultural forest sites.

Vegetation composition clearly differed between ancient and post-agricultural forest plots. A DCA on vegetation data markedly separated both forest ages along the first axis (Appendix 3.1). From the DCA it was also clear that the herb species *U. dioica* had its optimum in post-agricultural forest plots; average cover in ancient (1 %) and post-agricultural (29 %) forest plots was significantly different (Mann-Whitney Z = -6.63, $P < 0.001$). Average sum of cover of herb layer species tended to be higher in ancient (67 %) compared to post-agricultural (54 %) forest plots, but this difference was marginally significant (Mann-Whitney Z = -1.95, $P = 0.050$).

Table 3.1 Soil properties (0–10cm depth) of experimental sites in 2007. All soil nutrient concentrations (P, K^+, Mg^{2+} and Ca^{2+}) are given in mg/100 g dry soil.

Soil property	ancient 1	ancient 2	Post-agric. 1	Post-agric. 2	Post-agric. 3	Post-agric. 4
Texture†	loamy sand	sandy loam	loamy sand	loamy sand	loamy sand	fine sand
pH-KCl	6.5	4.7	4.5	4.0	4.2	4.3
C (%)	3.8	3.5	3.1	3.0	3.3	3.1
P	0.7	0.8	8.5	8.9	6.8	5.5
K^+	7.3	9.6	12.5	17.1	9.1	7.3
Mg^{2+}	6.5	13.0	9.4	5.5	7.0	10.3
Ca^{2+}	360.3	245.7	110.3	68.9	122.6	118.2

†: texture determined in 2001

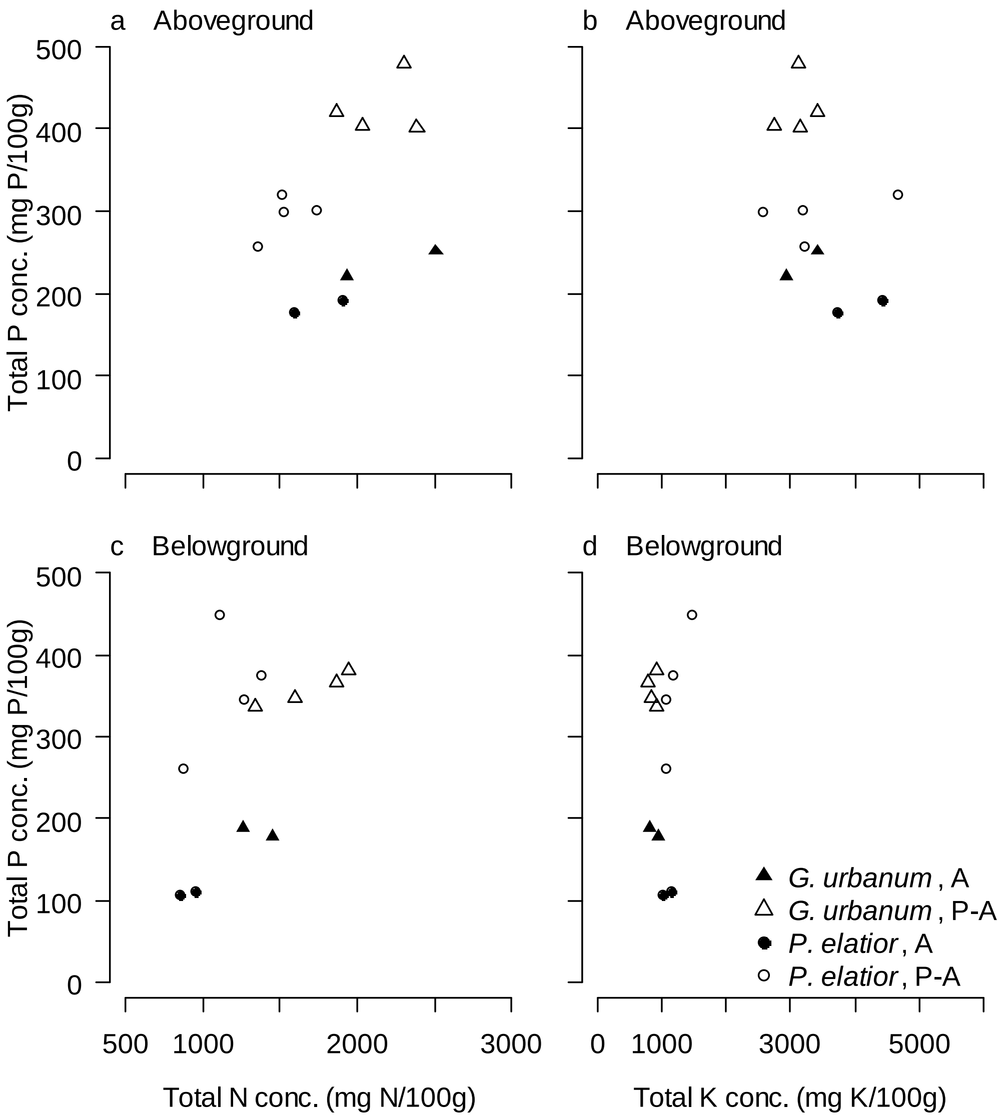


Fig. 3.2 Total P versus N (left) and total P versus K (right) plant tissue concentrations in the aboveground biomass (a, b) and the belowground biomass (c, d) of *Geum urbanum* (triangles, Δ) and *Primula elatior* (circles, o). Filled symbols represent samples from ancient forest sites (‘A’), open symbols from post-agricultural forest sites (‘P-A’).

3.2 Recruitment, longevity and performance

In seeded plots, the average number of adults was significantly higher in post-agricultural forest and in plots initially cleared of surrounding vegetation for *G. urbanum* (Fig. 3.3, Table 3.2a). In ancient forest plots that were not cleared, no adult of *G. urbanum* could recruit from seeds. A significant interaction between forest age and vegetation clearing resulted from a stronger clearing effect in ancient forest plots compared to post-agricultural forest plots. The average number of adults of *P. elatior* that could recruit from seeds was higher in ancient compared to post-agricultural plots (Fig. 3.3, Table 3.2a).

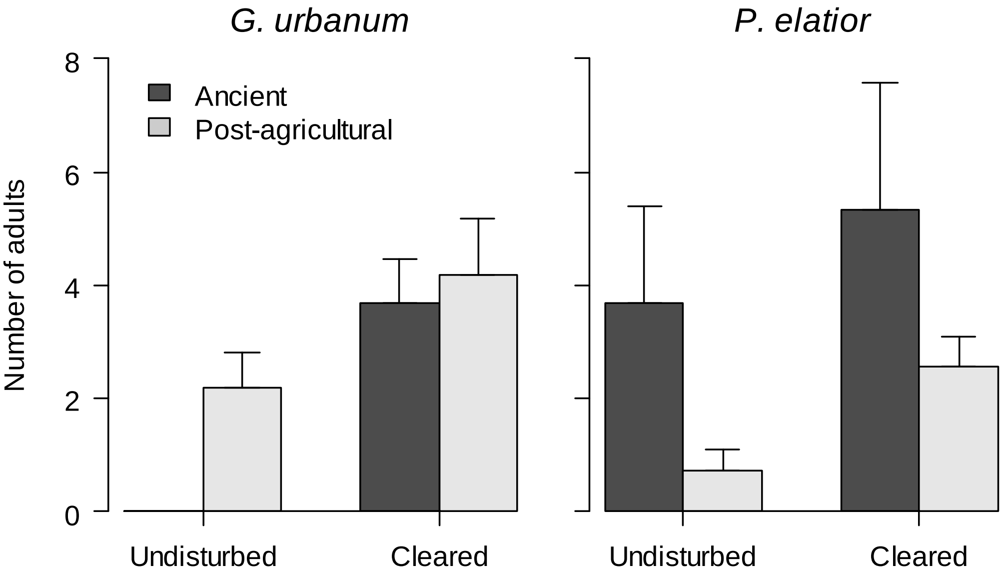


Fig. 3.3 Mean number of adults in seeded plots in 2007 (n = 36 plots per species). Seeds of *Geum urbanum* and *Primula elatior* were introduced in 1999 in ancient and post-agricultural forest, with or without the surrounding vegetation cleared. Error bars represent standard error. Statistical differences between groups are given in Table 3.2a.

In plots where individuals were introduced as adults the results were largely analogous. For *G. urbanum*, vegetation clearing had a stronger effect in ancient than in post-agricultural forests, and *P. elatior* had greater numbers of adults in ancient compared to post-agricultural forests (Fig. 3.4, Table 3.2b). In these plots, a distinction could be made between the originally introduced adult individuals and new recruitment of individuals. *Geum urbanum* showed a high level of new recruitment in all plots, except for ancient forest plots with continuous vegetation cover (undisturbed). Mortality in these plots was just counterbalanced by the recruitment of new individuals leaving the total number of individuals per plot at a constant level. New establishment of *P. elatior* was lower in post-agricultural forest; recruitment of new individuals did not counterbalance mortality in post-agricultural forest plots with continuous vegetation cover and the number of adults per plot decreased below the initial number of introduced adults.

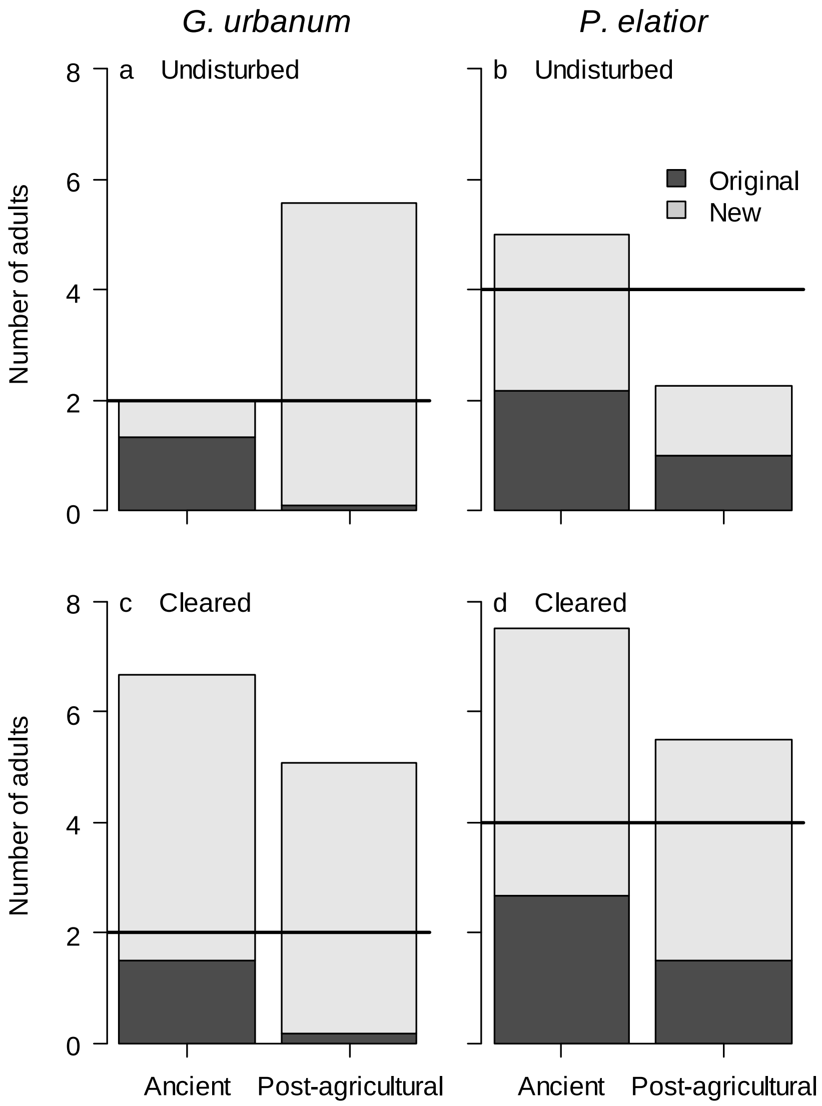


Fig. 3.4 Mean number of adults per plot in 2007 (n = 36 plots per species). Adults of *Geum urbanum* and *Primula elatior* were introduced in ancient and post-agricultural forest in 1999, with or without the surrounding vegetation cleared. Distinction was made between originally introduced and newly established individuals. The horizontal line indicates the number of individuals that were introduced in 1999. Statistical differences between groups are given in Table 3.2b.

Table 3.2 The effect of former land use and vegetation clearing on the mean number of adult individuals per plot eight years after the species were introduced by (a) seed sowing or (b) planting adult individuals. See methods for details on the model building.

Source of variation	*Geum urbanum*			*Primula elatior*		
	Effect	z/t	*P*	Effect	z/t	*P*
(a) Introduced as seeds						
Land use	+	2.326	0.020	–	-3.112	0.002
Cleared	+	3.007	0.003		1.351	0.177
Land use × cleared		-2.230	0.026		1.927	0.054
(b) Introduced as adults						
Land use	+	2.547	0.011	–	-1.661	0.097
Cleared	+	3.650	< 0.001	+	1.720	0.086
Land use × cleared		-3.387	< 0.001		1.368	0.171

Notes: Effects were tested with Generalized Linear Mixed Models with poisson errors and log link function including 'Site' as random effect term. Effects are reported if $P < 0.1$. 'Land use' and 'Cleared' were coded as binary variables with 0 = ancient forest or undisturbed and 1 = post-agricultural forest or cleared. Thus a positive effect of 'Land use' or 'Cleared' indicates higher numbers of adults in post-agricultural or cleared plots, respectively.

The survival of transplants between 2000 and 2007 was lower in post-agricultural compared to ancient forest sites for both species (Fig. 3.5, Table 3.3). Moreover, a significant forest age × year interaction for *G. urbanum* indicated that the decline in the number of surviving individuals was faster in post-agricultural forest as opposed to ancient forest. Initial clearing of the surrounding vegetation did not affect longevity.

Table 3.3 The effect of former land use and vegetation clearing on the proportional survival of *Geum urbanum* and *Primula elatior* between 2000 and 2007. See methods for details on the model building.

Source of variation	*Geum urbanum*		*Primula elatior*	
	z	*P*	z	*P*
Year	-3.343	0.001	-10.268	< 0.001
Land use	4.242	< 0.001	-2.138	0.033
Cleared	-0.128	0.898	1.573	0.116
Year × Land use	-4.251	< 0.001		

Notes: Effects were tested with Generalized Linear Mixed Models with binomial errors including 'Site' and 'Subplot' as non-nested random effect terms.

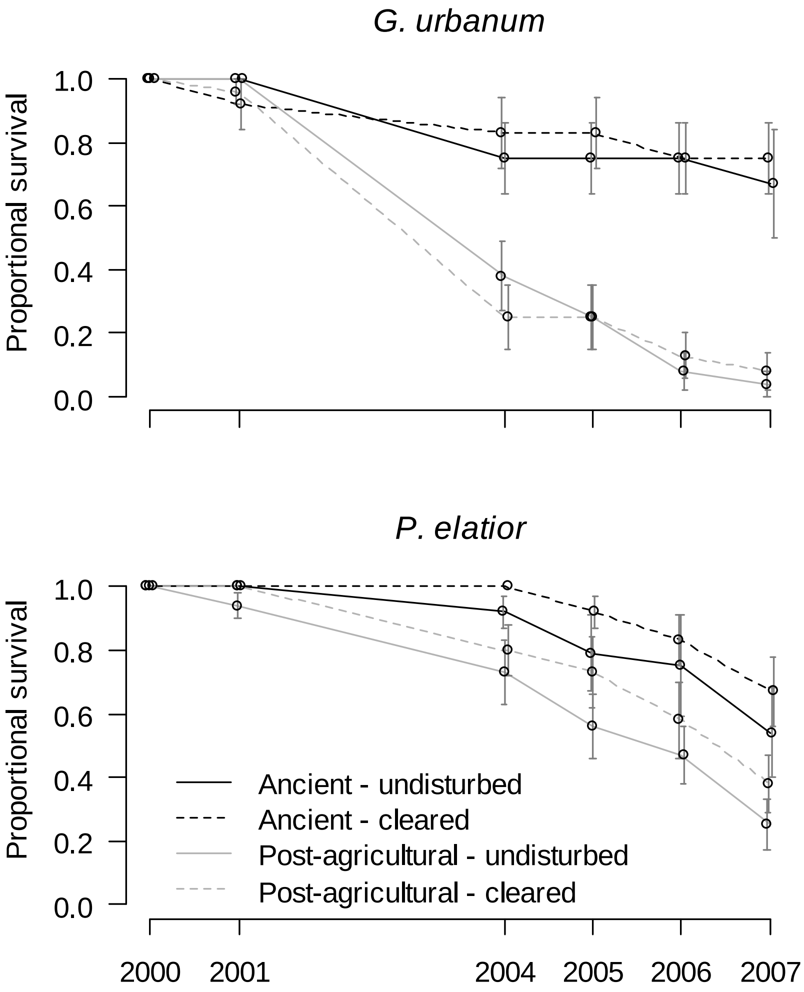


Fig. 3.5 Longevity by means of proportional survival of transplanted adult individuals between 2000 and 2007. Adults of *Geum urbanum* and *Primula elatior* were introduced in 1999 in ancient and post-agricultural forest sites, with or without the surrounding vegetation cleared (n = 36 plots per species). Survival of individuals was not monitored during 2002 and 2003. Standard errors are represented by gray error bars (jittered). Statistical results are given in Table 3.3.

Average plant performance per plot of *G. urbanum* was significantly better in post-agricultural forest as well in 2001 as in 2007 (Fig. 3.6, Table 3.4). By contrast, *P. elatior* performed better in post-agricultural forest and in competition free plots in 2001, but these differences were no longer found in 2007 (Fig. 3.6, Table 3.4). In 2001, the index for plant performance was affected by all five measured plant characteristics. In 2007, vegetative plant characteristics were, however, marginally correlated with this index so that plant performance should be mainly interpreted as generative adult performance (Appendix 3.2).

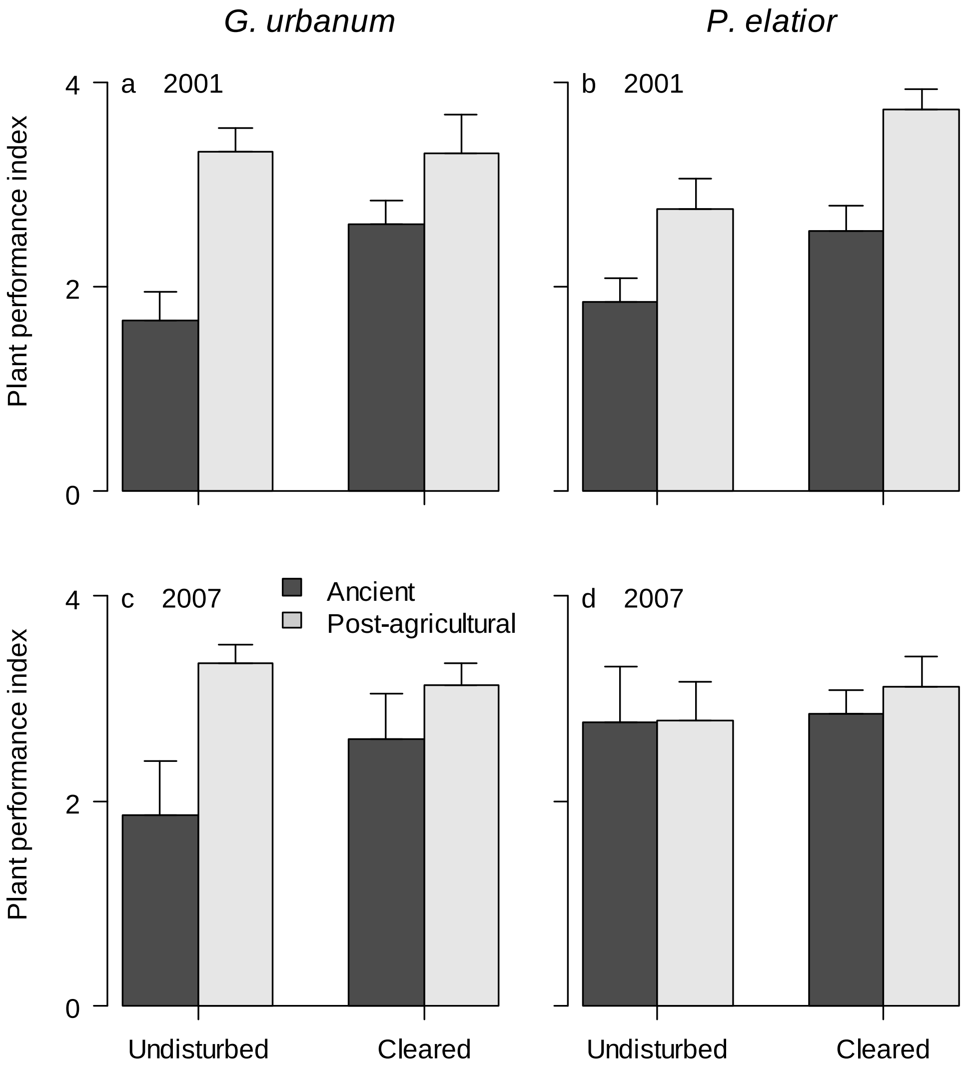


Fig. 3.6 Mean plant performance index (PPI) per plot for *Geum urbanum* and *Primula elatior* in 2001 and 2007. Error bars represent standard error. Statistical differences between groups are given in table 3.4.

Table 3.4 The effect of former land use and vegetation clearing on the mean plant performance per plot of adult individuals (*Geum urbanum*, *Primula elatior*) in 2001 and 2007.

Source of variation	*Geum urbanum*			*Primula elatior*		
	Effect	z/t	*P*	Effect	z/t	*P*
(a) Plant performance in 2001						
Land use	+	4.427	0.011	+	2.749	0.051
Cleared	+	2.639	0.015	+	2.054	0.050
Land use × cleared		-2.325	0.030		0.102	0.920
(b) Plant performance in 2007						
Land use	+	2.359	0.078		0.003	0.998
Cleared	+	2.101	0.044		0.599	0.555
Land use × cleared		-1.840	0.076		0.144	0.887

Notes: Effects were tested with Generalized Linear Mixed Models with normal errors including 'Site' as random effect term; the mean plant performance was log-transformed. Effects are reported if $P < 0.1$. 'Land use' and 'Cleared' were coded as binary variables with 0 = ancient forest or not cleared and 1 = post-agricultural forest or cleared. Thus a positive effect of 'Land use' or 'Cleared' indicates higher values in post-agricultural or cleared plots, respectively.

4 Discussion

Our general hypothesis – that differential effects of the habitat quality in post-agricultural forests on species recruitment and performance partially account for differences in colonization capacity – is generally supported by the data presented in this study. Post-agricultural forest sites were characterized by high P availability and dominance of competitive species, which differently affected recruitment success and adult performance of the slow colonizing forest herb *P. elatior* compared to the fast colonizing *G. urbanum*. Moreover, qualitative observations of two other experimentally introduced forest herbs with a different growth form (geophytes) that also show a contrasting colonization capacity are consistent with the observed patterns of these two hemicryptophytes. The expansion after introduction of the slow colonizing forest herb *A. nemorosa* was lower in post-agricultural forest compared to ancient forest, whereas the fast colonizing forest herb *R. ficaria* showed higher expansion in post-agricultural forest sites. In 2007, i.e., eight years after introduction as seeds or adults, *A. nemorosa* still occurred in 90 % of the ancient forest plots with an average cover of 19 % and only occurred in 41% of the post-agricultural forest plots with an average cover of 2 %. *Ranunculus ficaria*, on the other hand, was found in 98 % ancient and 95 % post-agricultural forest plots with 16 % and 27 % average cover, respectively. These differential effects of land-use history on the demography of the introduced species nuance the earlier findings of Verheyen & Hermy (2004), who only studied germination, survival and performance during two years following the experimental introduction. The results presented here therefore stress the importance of long-term studies across multiple life stages in assessing the importance of establishment limitation for plant colonization.

4.1 The nature of establishment limitation

Environmental constraints on the demography of species colonizing post-agricultural forests compared to ancient forest are mostly expected when there are marked differences in the (a)biotic environmental conditions they provide. Since differences in habitat quality between ancient and post-agricultural forests show large variations among regions and study sites (Flinn & Vellend 2005; Chapter 1), a first critical step in the assessment of establishment limitation is the characterization of the (a)biotic conditions; i.e., is there reason to expect reduced establishment?

Among the measured variables, P availability represented a key difference in habitat quality between ancient and post-agricultural forest sites in our study area (Table 3.1). The topsoil layer contained up to ten times more plant available P in post-agricultural forest (cf. Verheyen & Hermy 2004). Similar higher P availability as a legacy of former agricultural land-use was reported elsewhere (e.g., Koerner et al. 1997; Dambrine et al. 2007), but is particularly representative of afforestations performed during recent decades (e.g., Verheyen et al. 1999; De Keersmaeker et al. 2004). Indeed, afforestations that date from the period before the intensive use of fertilizers often turn out to be very similar in nutrient status compared to ancient forest sites (e.g., Motzkin et al. 1996; Graae et al. 2004; Flinn & Marks 2007). In addition, the difference in soil P availability was consistently reflected in the plant tissue nutrient concentrations (Fig. 3.2). A similar relation between soil P and plant tissue P was recently found by Falkengren-Grerup et al. (2006), albeit much less pronounced. Given its persistent higher availability and effect on plant uptake, P is likely to be a key factor to explain establishment limitation in these post-agricultural forest sites (see also Chapters 4 & 5).

We expect P availability to have both beneficial and detrimental effects on the recruitment and performance of forest plants. Hipps et al. (2005), for instance, found large positive growth responses of three forest herbs to supplied P concentrations in the absence of competition. Direct detrimental effects of P on plant fitness, on the other hand, are unlikely as total plant P concentrations were far below toxic levels of 1000 mg/100g dry biomass

(Marschner 1995). Rather, the vigorous growth of competitive species profiting from increased P availability, especially the P limited *U. dioica* (Pigott 1971; Hipps et al. 2005; Taylor 2009), is a more likely mechanism causing establishment limitation (Honnay et al. 1999; De Keersmaeker et al. 2004). Hipps et al. (2005) found that superior shoot growth and leaf development of *U. dioica* at high P availability restricted the growth of the two slow colonizing forest herbs *Veronica montana* L. and *Lamium galeobdolon* L. Our vegetation survey of all experimental plots indeed indicated vigorous growth of *U. dioica* in the post-agricultural forest plots. Exclusion of forest plants by competitive species such as *U. dioica* (Al-Mufti et al. 1977; De Keersmaeker et al. 2004) would therefore more than likely represent the link between P availability and establishment limitation. Other factors such as microtopography, herbivory and altered mycorrhizal associations could additionally underlie environmental limitation, but they were not explicitly tested here.

4.2 Recruitment, longevity and persistence

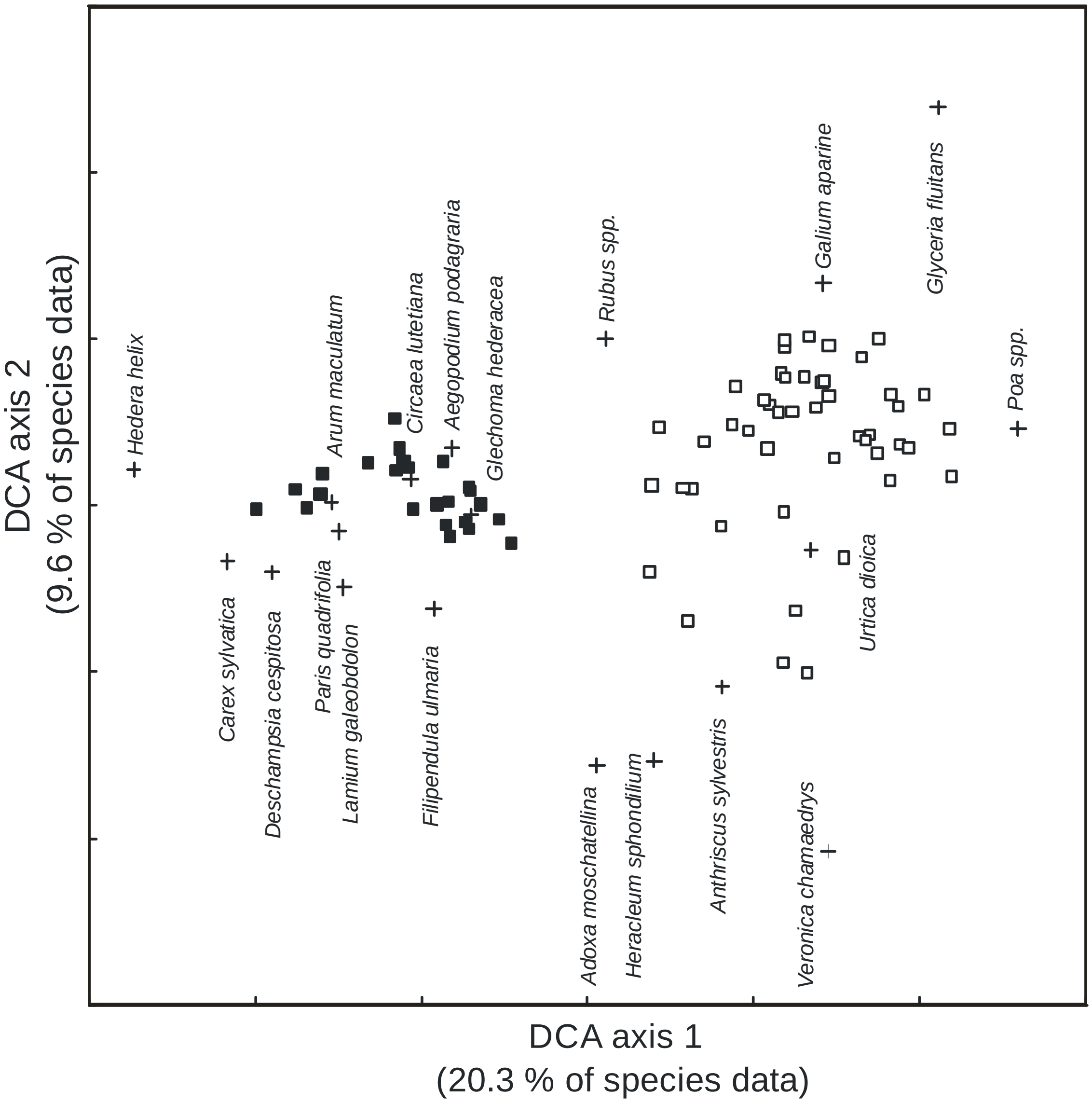
Initially, only clearing of the surrounding vegetation and not forest age affected germination after seed introduction (Verheyen & Hermy 2004). It was concluded that *G. urbanum* and *P. elatior* needed a spatial regeneration niche in ancient and post-agricultural forests – microsite limitation *sensu* Eriksson & Ehrén (1992) – and were not otherwise environmentally limited. Six years later, however, forest age shows a significant and differential effect on recruitment success, suggesting species specific environmental constraints on life stages after germination. *Geum urbanum* showed higher recruitment in post-agricultural forest, whereas *P. elatior* had higher recruitment success in ancient forest (Fig. 3.3). Just like Flinn (2007) recently demonstrated for three fern species, these species-specific recruitment rates differ as predicted by the species' contrasting distributions. Furthermore, *G. urbanum* established primarily in cleared plots in ancient forests, but unlike *P. elatior*, the habitat provided by post-agricultural forests enabled this species to become less dependent on understory gaps for recruitment. Higher adult performance in post-agricultural forest sites, including the number of inflorescences and flowers, regardless of competition by the surrounding vegetation presumably accounts for this recruitment success. Unlike similar seed introduction experiments by Donohue et al. (2000) and Graae et al. (2004), which were short term and only monitored recruitment to a seedling stage, we found species-specific establishment limitation on the post-seedling stages of recruitment. Looking at recruitment as a bottleneck process, environmental constraints on only one demographic stage is needed to limit a species' colonization capacity, regardless of its performance in other stages. For instance, Donohue et al. (2000) found that, though its performance was high in post-agricultural forests, an intrinsic slow growth rate and limited seedling establishment of *Gaultheria procumbens* L. restricted the colonization of this herb to post-agricultural forest sites. Similarly, Endels et al. (2004) found higher performance of *P. elatior* and *G. urbanum* in post-agricultural compared to ancient forest habitats, but suggested that low seedling survival accounted for a large discrepancy between the high densities of seedlings and low densities of adults. Enhanced or equal adult performance observations in post-agricultural forest sites (see also Fig. 3.6; Verheyen & Hermy 2004; Fraterrigo et al. 2006; Flinn 2007) thus not necessary exclude environmental restrictions on the colonization process. Our results underscore a comment made in similar introduction experiments that, given the typically long life spans of forest plants (cf. Ehrlén & Lehtilä 2002), results on initially successful seedling emergence can not rule out that recruitment is hampered by habitat quality in subsequent stages (Gustafsson et al. 2002; Graae et al. 2004). We demonstrate that understory gap availability – a result of clearing vegetation – governs seedling emergence (cf. Eriksson & Ehlén 1992), but that the relative importance of other habitat characteristics increases when considering mortality across more than a single life stage, as recently suggested by Clark et al. (2007).

Adults clearly showed lower longevity in post-agricultural forest sites (Fig. 3.5). This forest age effect on longevity was originally not observed two years after introduction (Verheyen & Hermy 2004). The conclusion that habitat quality was not a limiting factor should therefore be revised in light of these longer term results. The same conclusion was drawn by Gustafsson et al. (2002) who found transplants of the forest herb *Dentaria bulbifera* L. to have lower survival in originally unoccupied plots. Among other (a)biotic limitations, high interspecific competition in post-agricultural forest sites resulting from the vigorous growth of competitive species is a sound explanation to account for the observed lower survival, especially for *P. elatior*. Indeed, the growth of the competitive herb *U. dioica* (discussed above) was an important factor limiting the colonization of *P. elatior*, among other slow colonizing forest plants, in the forest Muizenbos (Verheyen & Hermy 2001a). Our performance observations confirm this finding because *P. elatior*, unlike *G. urbamum*, seems to suffer from competition as it could not sustain its initial higher performance in post-agricultural forest sites in the longer term (Fig. 3.6). Furthermore, it is not unlikely that life cycles of both forest herbs are much shorter in post-agricultural forest sites, which would indicate a trade-off between growth and longevity. For tree species, for instance, the suggestion has been made that slow growth corresponds to prolonged life spans (Ward 1982; Peñuelas 2005). Fraterrigo et al. (2006) studied the allocation and growth of forest plants in ancient and post-agricultural forest stands and in fact found growth to be enhanced by higher nutrient levels, including P, in post-agricultural sites. Similar results were obtained in heath communities where experimental nutrient enrichment, especially P, caused plants to speed up their life cycles (Heddle & Specht 1975).

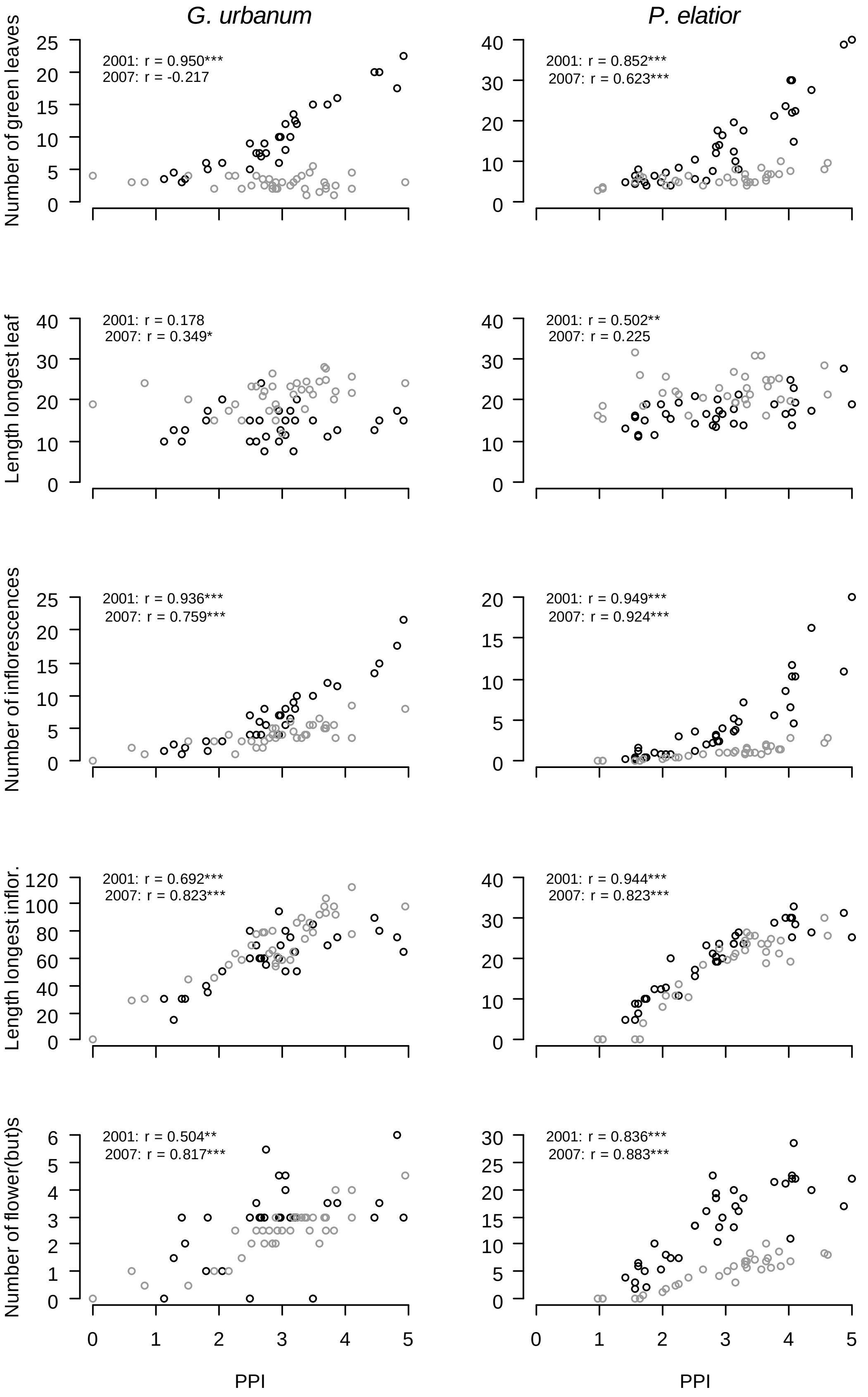
Since longevity of individuals is a major determinant for population growth of long-lived forest plant species (Silvertown et al. 1993), this lower longevity can seriously slow the expansion of populations if it is not counterbalanced by the establishment of new individuals. The key question regarding the persistence of the introduced populations in the long term is thus whether they could counterbalance increased mortality by new establishment. In accordance with the observations on recruitment in seeded plots, *G. urbanum* overcompensated mortality by new establishment in the post-agricultural forest sites so it could successfully expand. *Primula elatior*, on the other hand, was less successful and dropped below the initial number of introduced individuals in plots with continuous vegetation cover (Fig. 3.4). The persistence of *P. elatior* in the long term, even after by-passing dispersal limitation through introduction, is therefore questionable.

Both at the forest (Verheyen & Hermy 2001a,b) and the landscape scale (Verheyen et al. 2003b), a dominant effect of spatio-temporal isolation on distribution patterns of the slow colonizing forest herbs *P. elatior* and *A. nemorosa* has clearly indicated that the colonization of these herbs is primarily limited by dispersal. The colonization success of the fast colonizing *G. urbanum* and *R. ficaria*, on the other hand, was not significantly hampered by dispersal. After eliminating dispersal constraints through seed introduction, results from this study now add to this that the slow colonizers are additionally limited by the habitat quality provided by the post-agricultural forest sites whereas the fast colonizers in fact could profit from it. In this way, the overrepresentation of good dispersers in post-agricultural forests is accentuated by additional environmental effects on recruitment, longevity and performance. These experimental results thus confirm the suggestion that the colonization process, at least for these species, is a two-stage process in which diaspore availability creates an initial ecological filter followed by species specific environmental constraints at later stages (Verheyen et al. 2003b). Furthermore, this empirical study provides a useful test of the current theoretical debate on the underpinnings of community assembly as it supports the view that ‘dispersal assembly’ (Hubbell 2001) and ‘niche assembly’ (Chase & Leibold 2003) are non-exclusive mechanisms shaping the composition of a plant community. A key question now concerns the degree to which traits that influence dispersal and establishment are correlated across species.

Appendix 3.1 Detrended correspondence analysis on log-transformed cover data of understorey species (+) in ancient (full square) and post-agricultural forest plots (open square) in 2007. The introduced species (*A. nemorosa, R. ficaria, P. elatior* and *G. urbanum*) were not included in this analysis. Only species with a cumulative fit > 5 % are indicated in the diagram.



Appendix 3.2 Spearman rank correlations between the plant performance index (PPI, first principal component axis derived from five plant characteristics) and the individual plant characteristics. o: 2001; o: 2007; NS: not significant; *: $P < 0.05$; **: $0.001 < P \leq 0.01$; ***: $P \leq 0.001$



CHAPTER 4 – FORMER LAND USE AFFECTS THE NITROGEN AND PHOSPHORUS CONCENTRATIONS AND BIOMASS OF FOREST HERBS: A FIELD STUDY

AFTER: BAETEN L, VERSTRAETEN G, DE FRENNE P, VANHELLEMONT M, WUYTS K, HERMY M & VERHEYEN K. FORMER LAND USE AFFECTS THE NITROGEN AND PHOSPHORUS CONCENTRATIONS AND BIOMASS OF FOREST HERBS. *PLANT ECOLOGY*, ACCEPTED.

Abstract

The colonization rates of understorey plants into forests growing on former agricultural land differ remarkably among species. Different dispersal and recruitment largely account for the contrasting colonization rates, but different effects of the soil legacies of former agricultural land use on plant performance may also play a role. Seven slowly to fast colonizing forest herbs were sampled in paired post-agricultural and ancient forest stands to study whether land-use history has an effect on the aboveground nutrient concentrations (N, P and N:P ratios) and biomass of forest herbs and, if so, whether slow and fast colonizing species respond differently. Results showed that P concentrations were significantly affected by former land use with higher concentrations in the post-agricultural stands. N concentrations were unaffected and N:P ratios were significantly higher in the ancient stands. Nutrient concentrations varied considerably among species, but the variation was unrelated to their colonization capacity. Six out of the seven species had higher biomass in the post-agricultural stands relative to the ancient stands and the degree to which the species increased biomass was positively related to their colonization capacity, i.e., the fast species showed the strongest increase. Such different response to past land use may contribute to the contrasting colonization capacity of forest plants. Land-use history thus affected both the nutrient concentrations and biomass of forest herbs and only the biomass response was related to colonization capacity.

1 Introduction

The availability of nitrogen (N) and phosphorus (P) to terrestrial vegetation profoundly influences several aspects of community dynamics such as plant performance, plant species interactions and multitrophic interactions (e.g., Aerts & Chapin 2000; Güsewell 2004; Craine 2009). Consequently, environmental changes that alter the levels of N and P supply may have a significant impact on the structure and composition of plant communities. Determining the response of plants to those novel environmental conditions is therefore one of the challenges of applied ecological research.

For forest understorey communities, land-use change forms among the most radical and large-scale environmental changes in much of Europe and eastern North-America. In many landscapes, large parts of the present-day forest area have not been continuously forested during the past decades or centuries, but were in fact established on former agricultural land in a recent or distant past ('post-agricultural' forest) (reviews: Flinn & Vellend 2005; Hermy & Verheyen 2007; Chapter 1). Previous studies found that the performance of forest plants may considerably differ between post-agricultural forests and forests without historical records of past agricultural use ('ancient' forest) (Endels et al. 2004; Verheyen & Hermy 2004; Vellend 2005; Fraterrigo et al. 2006; Chapter 3). Those performance differences, which can be either enhanced or depressed in post-agricultural forest, may be related to the soil nutrient legacies of the historical land use. The past agriculture may have a persistent impact on the present-day soil N and P state: the soil P is generally higher in previously fertilized forest compared to ancient forest and the soil N may be lower, equal or higher (e.g., Koerner et al. 1997; Verheyen et al. 1999; De Keersmaeker et al. 2004; Falkengren-Grerup et al. 2006; Dambrine et al. 2007; Table 1.1). While pot experiments have demonstrated that some forest plants may increase their performance in response to increased N and P availability (Pigott & Taylor 1964; Elemans 2004; Hipps et al. 2005; see also Chapter 5), the results still largely need to be extended to field conditions (but see Falkengren-Grerup et al. 2006; Fraterrigo et al. 2009).

Forest plant species differ considerably in their capacity to colonize post-agricultural forests. Some species already appear soon after canopy closure, but for others it may take several decades to centuries. Dispersal constraints initially cause the limited colonization abilities (reviews: Verheyen et al. 2003c; Vellend et al. 2007; Chapter 1), but effects of past land use on plant performance may also play a role (Vellend 2005; Chapter 3). Within the perspective of the potentially altered N and P supply in post-agricultural forests, we wondered whether differences in the colonization capacity among forest plants were associated with variation in their plasticity in response to N and P. If fast colonizing species also tend to increase their performance more at increased nutrient levels compared to slow colonizers this would imply that the community recovery in post-agricultural forests is not only controlled by dispersal, but also by the soil nutrient legacies of past land use.

Here we determined the effect of former agricultural land use on the aboveground nutrient concentrations (N, P and N:P ratios) and total biomass (as a measure of performance) of seven common forest herbs in valley forest stands in northern Belgium. We used the two introduction experiments of Chapter 2 and 3 in which a set of slowly colonizing forest herbs were introduced into post-agricultural forest ten and eight years ago. In this way, we could sample both slowly colonizing species (introduced) and fast colonizing species (resident community). We hypothesised that (i) land-use history has a significant effect on the nutrient concentrations and biomass of the forest herbs, (ii) there is considerable variation among species and (iii) the inter-specific variation is related to the colonization capacity of species with the fast colonizing species showing the most plastic response.

2 Methods

2.1 Study sites and soil samples

Plant tissue of seven forest herbs was collected in post-agricultural and continuously forested ancient forest stands (*sensu* Hermy et al. 1999) at three different forest sites in northern Belgium: two separate introduction experiments and one additional site with spontaneous colonization. All sites were on moist, eolian, loamy soils that supported a mesotrophic vegetation characteristic of valley forest. The main canopy species were *Populus* × *canadensis*, *Fraxinus excelsior* L. and *Corylus avellana* L. Regional average (1971–2000) annual temperature is 10.1 °C and the average annual precipitation amounts to 820 mm (Royal Meteorological Institute, www.kmi.be).

The first introduction experiment was set up in the forest ‘Muizenbos’ in 1999 (51.197 °N, 4.566 °E). Experimental populations of *Primula elatior* Hill, *Anemone nemorosa* L., *Ranunculus ficaria* L. and *Geum urbanum* L. were established in two ancient stands (forest for more than 145 year based on land-use maps) and four post-agricultural stands (*c.* 15 year old) on former heavily fertilized grassland. In each stand, every species was introduced by sowing seeds in six 0.5 m × 0.5 m plots or planting adults or ramets (for geophytes) in six similar plots. The twelve plots were regularly distributed in a 12 m × 12 m fenced area. For the present study, we sampled the two ancient stands and two of the post-agricultural stands. More details on the design of the experiment and population dynamics are reported in Verheyen and Hermy (2004) and in Chapter 3.

The second introduction experiment was initiated in a forested landscape 20 km east of Brussels in 2002 (‘Doode bemde’ nature reserve; 50.827 °N, 4.642 °E). Six forest herbs, including *P. elatior, A. nemorosa* and *G. urbanum*, were introduced in two valley forest stands that were established on former agricultural land between 1909 and 1940. In each stand, the species were sown into twelve 0.5 m × 0.5 m plots that were also regularly distributed in a 12 m × 12 m fenced area. For the present study, we only sampled plants from one of the stands because the canopy of the other stand was dominated by *Alnus glutinosa* (L.) Gaertn. (a N-fixing species), which could alter the soil N concentrations. Because the species were only introduced into post-agricultural forest, we also sampled plant material in a 12 m × 12 m area in a nearby ancient stand (420 m away). The ancient stand had been forest for more than 224 year (based on Verheyen et al. 2003b) and was characterized by a similar soil and canopy. Chapter 2 gives more details on the design of this introduction experiment.

Finally, we also sampled plants in a post-agricultural and adjacent ancient stand of a third forest site ‘Aelmoeseneiebos’ (50.975 °N, 3.795 °E). Unlike the other two sites, the slowly colonizing forest species had not been introduced here but they had colonized spontaneously. Colonization probably occurred from the source populations in three adjacent ancient forest stands. The post-agricultural stand was established in 1968 on former grassland, the ancient stand had been forested at least since 1775. At this forest site, we also sampled plant tissue in 12 m × 12 m areas.

At each forest stand, we took five soil samples (0–10 cm depth) and merged them into a single mixed soil sample. The mixed samples were dried for *c.* 48 h at 40 °C and analysed for pH-KCl, total P (nitric-perchloric acid digestion) and ammonium acetate-EDTA extractable P (as an indicator for plant available P). The P concentrations were measured colorimetrically by the molybdate method of Scheel (1936). Total soil N and C were measured by dry combustion at 850 °C using an elemental analyzer Vario MAX CNS, Elementar.

2.2 Study species, sampling and chemical analyses

Seven common forest herbs that naturally occur in mesotrophic valley forest and represent a range of colonization abilities were sampled (Table 4.1). In addition to the species that were introduced in the experiments (*A. nemorosa, P. elatior*, *G. urbanum* and *R. ficaria*), we also sampled plant material from spontaneous populations of three other forest herbs with moderate (*Circaea lutetiana* L.) and high (*Aegopodium podagraria* L.*, Urtica dioica* L.) colonization abilities. In this way, we could determine whether fast colonizing forest plants may respond differently to the former land use in terms of N and P uptake and biomass production compared to slow colonizers. The colonization capacities of the forest species were based on a review study by Verheyen et al. (2003c), in which a species-specific colonization capacity index (CCI) was calculated based on the species' affinity to ancient forest across 12 studies in north-western Europe. The CCI ranges between -100 (fast colonizer) and +100 (slow colonizer).

In each forest stand, we collected the total aboveground biomass of maximum five random individuals of each species (three or four individuals in a few cases). For the geophytes, we sampled a number of ramets in five separate patches (to obtain enough biomass for analysis). The average number of ramets (± SE) collected per patch was 13.9 ± 3.4 for *A. nemorosa*, 5.4 ± 1.3 for *C. lutetiana* and 8.5 ± 3.0 for *R. ficaria*. Sampling occurred following peak flowering, i.e., in the first week of May for the three vernal species and in the first week of July for the other species. Next, the collected individuals or ramets were dried to constant weight for *c.* 48 h at 70 °C. The biomass of each sample was weighted (± 1 mg), and for the geophyte samples, the average ramet biomass was calculated (total mass/number of ramets). Plant material was analysed for total N concentrations (mg N/100 g dry biomass) with the Kjeldahl method. Total P concentrations (mg P/100 g dry biomass) were measured colorimetrically by the molybdate method (Scheel 1936) following a di-acid digestion (HNO_3 65%, $HClO_4$ 70%, 5:1).

Table 4.1 Life form, phenology and colonization capacity of the sampled forest herbs. The colonization capacity is the colonization capacity index from Verheyen et al. (2003c) and ranges from +100 (slow colonization into post-agricultural forest) to -100 (fast colonization).

Species	Family	Life form	Leaf phenology	Colonization capacity index
Anemone nemorosa	Ranunculaceae	Geophyte	Vernal	77
Primula elatior	Primulaceae	Hemicryptophyte	Vernal-aestival	67
Circaea lutetiana	Onagraceae	Geophyte	Aestival	67
Ranunculus ficaria	Ranunculaceae	Geophyte	Vernal	11
Geum urbanum	Rosaceae	Hemicryptophyte	Winter green	-33
Aegopodium podagraria	Apiaceae	Hemicryptophyte	Aestival	-45
Urtica dioica	Urticaceae	Geophyte/ Hemicryptophyte	Aestival	-71

2.3 Data analysis

We applied Linear Mixed Models to account for the variation in N and P concentrations and N:P ratios among (1) the forest sites, (2) the forest stands nested within sites and (3) among the different species. The models tested the effect of three explanatory variables: the land-use history of the stands (post-agricultural versus ancient), the colonization capacity of the species (CCI index) and the biomass of the sampled individuals as a co-variable. Following Zuur et al. (2009) we first selected the optimal random effects structure based on a likelihood ratio test between models with the same fixed component (the three explanatory variables), but a different random component. The optimal model included 'Forest stand' and 'Species' as non-nested random effects, i.e., the variation among forest sites was not substantial. Next, we compared the model that included the three explanatory variables with a model that dropped one of those variables to test the significance of that particular variable (χ^2 test statistic, likelihood ratio test; Zuur et al. 2009). Finally, we also calculated the proportion of the total variation in the response variable that was associated with variation among different forest stands and among different species ('intraclass correlations') and the amount of this variation that was explained by the explanatory variables. All calculations were performed using the *lmer* function in the *lme4* package in R version 2.9.1 (R Development Core Team 2009; Bates & Maechler 2010).

The effect of 'Land use' (post-agricultural versus ancient) on the total aboveground biomass was tested in a similar way, but for each species separately because of their inherent differences in biomass related to growth form. The optimal random effects structure only included 'Forest stand', i.e., 'Forest site' did not account for additional variation. The significance of 'Land use' was also tested by comparing the mixed models with and without the factor based on a likelihood ratio test. Finally, we calculated the ratio between the average biomass of a species in the post-agricultural versus the ancient stand for each forest site. The average ratio for each species across the three sites was related to its colonization capacity index by means of linear regression.

3 RESULTS

Values for soil pH-KCl, total N and C were comparable between ancient and post-agricultural stands (Table 4.2). The average C:N ratio was 12.6 (± 0.2) in ancient and 11.5 (± 0.3) in post-agricultural stands. Land-use history did have an effect on the total and plant available soil P concentrations with higher levels in the post-agricultural stands.

Table 4.2 Chemical soil properties (0–10 cm) of the ancient (A) and post-agricultural stands (P-A) at the three forest sites where the forest herbs were sampled. We used an ammonium acetate-EDTA extraction of soil P as an indicator of plant available P (P-plant) and an extraction of the total P fraction (P-total).

	Muizenbos				Doode bemde		Aelmoeseneiebos	
	A	A	P-A	P-A	A	P-A	A	P-A
pH	6.4	4.7	4.5	4.2	6.0	5.4	4.5	5.5
P-total (mg/100g)	37.1	28.2	107.5	75.4	65.4	104.5	37.1	57.0
P-plant (mg/100g)	0.70	0.68	7.81	6.24	2.37	2.19	1.20	4.74
N (g/100 g)	0.25	0.19	0.24	0.21	0.31	0.36	0.37	0.22
C (g/100 g)	3.11	2.44	2.79	2.53	3.74	3.82	4.76	2.54

The total N concentration in the aboveground biomass of the seven forest herbs was unrelated to the former land use, colonization capacity and the biomass of the sampled plants (Table 4.3; Fig. 4.1a). The total plant P concentrations did differ between stands with different land-use histories. In post-agricultural forest stands, P concentrations were consistently higher in all species compared to ancient stands (Fig. 4.1b). Almost half of the variation in P concentrations (46.3 %) was associated with variation between stands, and land-use history explained 66.5 % of this variation. There was also considerable variation among species – 18.8 % of the variation in P concentration was attributable to species-specific differences – but this variation was unrelated to the colonization capacity of the species. The patterns for the N:P ratios were similar. Land-use history had a significant impact on the N:P ratios, which were consistently lower in post-agricultural versus ancient stands (Table 4.3; Fig. 4.1c). A large proportion of the total variation in N:P ratios was associated with variation between stands (59.4 %), and land use accounted for 69.1 % of this variation. Species-level variation was 15.5 %, but was unrelated to the colonization capacity of the species.

Table 4.3 Effects of Land use, Colonization capacity and Biomass on the N and P concentration and N:P ratio in seven forest herbs. The herbs were collected in the (experimental) populations of four ancient and four post-agricultural forest stands. See methods for details on model building.

	Effect	χ^2
N concentration		
Land use		0.419
Colonization capacity		0.385
Biomass		1.400
P concentration		
Land use	+	9.542**
Colonization capacity		2.315
Biomass		1.646
N:P ratio		
Land use	–	8.953**
Colonization capacity		0.613
Biomass		0.811

Notes: Effects were tested with Linear Mixed Models including 'Forest stand' and 'Species' as non-nested random effect terms; the N and P concentrations were transformed by the natural logarithm. The χ^2 statistic is based on a likelihood ratio test between a full model and a model without the factor. 'Land use' was coded as a binary variable (0: ancient; 1: post-agricultural), i.e., a positive effect indicates a higher value in post-agricultural versus ancient forest. **: $P < 0.01$

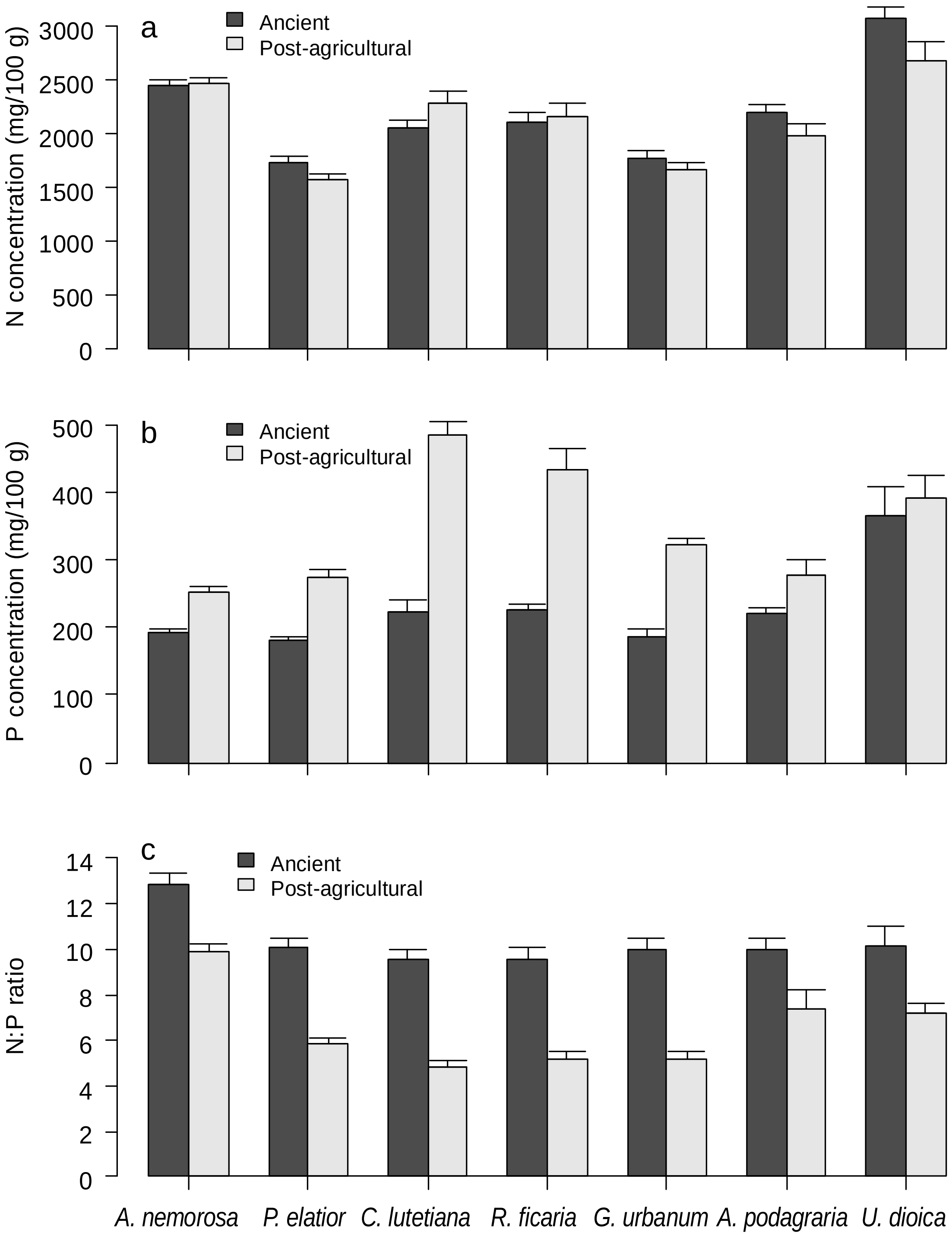


Fig. 4.1 Effect of land-use history on the N concentration (a), P concentration (b) and N:P ratio (c) for seven forest herbs (mean ± SE; n = maximum 40 per species). The herbs were collected in (experimental) populations of four ancient and four post-agricultural forest stands. The species were sorted according to their colonization capacity from left (slow colonizers) to right (fast colonizers). Results of the multilevel models are reported in Table 4.3.

.

Six out of the seven forest herbs tended to increase their biomass in the post-agricultural stands relative to the ancient stands (Fig. 4.2). For four species, the difference was significant at the 0.1 level. Only *A. nemorosa* did not show a higher average biomass per ramet in post-agricultural compared to ancient forest stands. *Urtica dioica*, on the other hand, showed the strongest response to former land use and increased its average biomass by a factor *c.* 4 in post-agricultural stands. Finally, the degree to which the species increased their biomass in post-agricultural versus ancient forest stands was significantly related to the colonization capacity index, i.e., the slowest colonizers (high CCI) showed the smallest relative increase in biomass (Fig. 4.3).

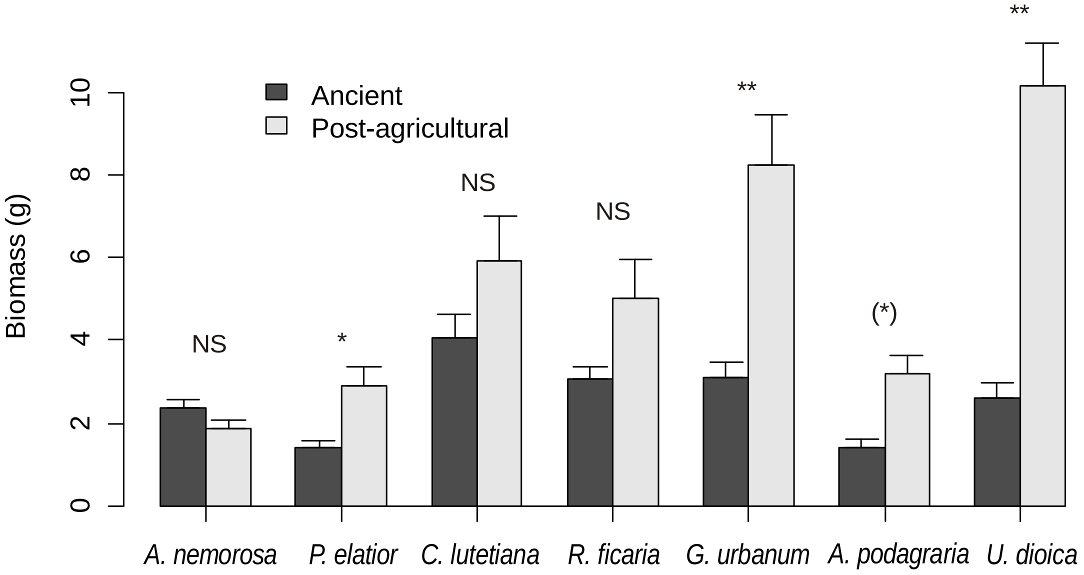


Fig. 4.2 Differences in the aboveground dry biomass of seven forest herbs in ancient and post-agricultural forest stands (mean ± SE; n = maximum 40 per species). The species were sorted according to their colonization capacity from slow colonizers (left) to fast colonizers (right). The biomass values for *A. nemorosa* and *R. ficaria* were rescaled to the biomass of 20 ramets and for *C. lutetiana* to 10 ramets. The effect of former land use was tested with multilevel models including 'Forest stand' as random effects term; NS: not significant; (*): $P < 0.1$; *: $P < 0.05$; **: $P < 0.01$.

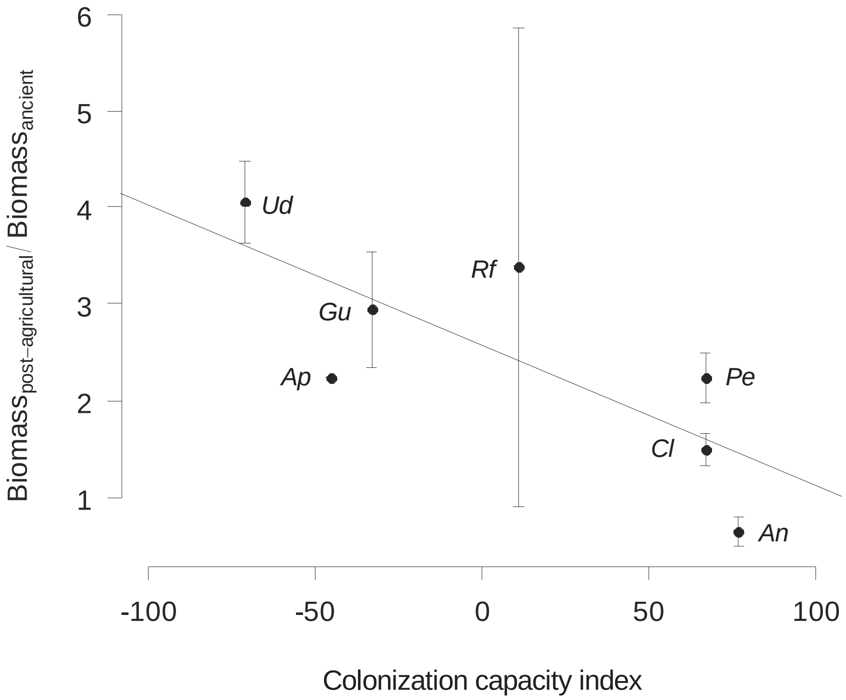


Fig. 4.3 Relation between the colonization capacity index (CCI) of the seven forest herbs and the mean ratio of their average biomass in post-agricultural versus ancient forest stands (averaged over the three forest sites). Slowly colonizing species have a high CCI value. Species names were abbreviated by the first letter of the genus and species name; error bars represent standard error. The slope of the linear relation was significantly different from zero (t = -2.714; $P < 0.05$) with $R^2 = 0.515$.

4 DISCUSSION

Studies that determined the impact of land-use history on the forest herb layer have mainly focused on the distributional patterns of forest plants and the degree to which dispersal and recruitment hamper their colonization (reviews: Flinn & Vellend 2005; Hermy & Verheyen 2007). Yet, once a forest plant has germinated, the former land use may also have effects on its performance (e.g., Endels et al. 2004; Verheyen & Hermy 2004; Vellend 2005; Fraterrigo et al. 2006; Chapter 3). The uptake of N and P forms a particular component of plant performance related to plant growth, but the issue has received little attention to date (Falkengren-Grerup et al. 2006; Chapter 3). Here we showed that land-use history significantly affected the aboveground P concentrations and biomass of seven forest herbs with higher concentrations and biomass in post-agricultural stands. Land-use effects on nutrient concentrations were similar for slow and fast colonizing species, but the fast colonizers tended to increase their biomass more in response to the former agriculture compared to the slow colonizers.

4.1 Land-use effects on plant nutrient concentrations

The seven forest herbs showed consistently higher P concentrations in their aboveground biomass in the post-agricultural stands compared to the ancient stands. The overall higher aboveground plant P concentrations indicates that former fertilization has increased the bio-available pool of soil P in the post-agricultural stands (Table 4.2), which is consistent with soil studies showing a remarkably persistent P enrichment in forests on former agricultural land (Koerner et al. 1997; Verheyen et al. 1999; De Keersmaeker et al. 2004; Falkengren-Grerup et al. 2006; Dambrine et al. 2007). Likewise, in Chapter 3 we showed that the contrasting land-use history of forest stands was reflected in the plant P concentrations of two forest herbs (*P. elatior, G. urbanum*). Furthermore, the plastic response of forest herbs to increased P availability we found here partly generalizes the results from pot experiments (Pigott & Taylor 1964; Hipps et al. 2005; see also Chapter 5) to field conditions and a larger set of species. The first hypothesis thus seems to hold regarding the plant P concentration. The N concentrations, on the other hand, did not differ between plants in post-agricultural and ancient forest stands (cf. Chapter 3). This lack of differentiation between the contrasting land-use histories may have principally two causes. First, the pool of plant available N might not strongly differ between the ancient and post-agricultural forest stands. Our soil data indicated that at least the total N pool does not depend on the historical land use of the sites (Table 4.2). Due to the high mobility and multiple loss pathways of mineral soil N (e.g., Schulze 2000), the legacy of past fertilization was probably only temporary, and the actual N state of the ecosystem is rather determined by factors such as soil and humus type, tree species composition and atmospheric N deposition loads (e.g., Compton et al. 1998; Gundersen et al. 2006). Second, the forest herbs may simply exhibit low plasticity in response to the variation in N availability because the nutrient is in excess at our sites. Previous experimental studies found that forest plants may increase their foliar N concentrations and aboveground biomass with N fertilization (e.g., Elemans 2004; Fraterrigo et al. 2009), but not if N was not limiting plant growth (Tessier & Raynal 2003). Explanations for a potentially excess N availability at our study sites are the inherently high N availability in alluvial forest systems (e.g., through fast litter decomposition, alluvial inputs), which is supplemented with a high total inorganic N throughfall deposition (34–46 $kg.ha^{-1}.yr^{-1}$ in the Flemish ICP Level II monitoring plots between 1993 and 2002, Neirynck et al. 2004).

There was substantial variation in plant N and particularly in plant P concentrations among the species (cf. second hypothesis), but the variation was unrelated to the capacity of the species to colonize post-agricultural forests. The third hypothesis is thus not supported by the data. In Chapter 5 we show similar patterns for P: in a pot experiment with four forest herbs both slowly and fast colonizing species may exhibit high plasticity in response to P addition. In the present study, the variation in N and P concentration among species was also not related to differences in life form or phenology. It is therefore difficult to extrapolate our results to other study sites, which concurs with the findings of Fraterrigo et al. (2006). Thus, the responses to P availability seem to be largely species specific and related to individual life histories.

Because former land use had an effect on the uptake of P in the aboveground biomass of the forest herbs, but not on N uptake, the N:P ratios in the studied forest herbs strongly differed between post-agricultural and ancient stands. As the N:P ratio of individual species was consistently lower in the post-agricultural stands, the vegetation-level N:P ratio is probably also reduced in those stands. Vegetation-level N:P ratios are commonly used as a practical tool to estimate the degree to which the community is limited by N availability (low N:P), by P availability (high N:P) or co-limited by both at intermediate N:P ratios (reviews: Tessier & Raynal 2003; Güsewell 2004). An important restriction to the application of N:P ratios to assess nutrient limitation, however, is that either N or P is a limiting resource for plant growth (Tessier & Raynal 2003). The relatively low N:P ratio in post-agricultural forest should therefore not be interpreted as an indication of N limitation at those stands, which would be very unlikely at our alluvial sites (see above). The increased P concentrations and altered N:P ratios in the herbaceous species may have important implications for some key components of the ecosystem functioning. For instance, the herbaceous layer accounts for a considerable proportion of the total annual foliar litter fall (on average 10–15 %) and this herbaceous litter forms a particularly labile fraction of fast decomposing material (overviews in Muller 2003; Gilliam 2007). The higher P concentrations in the aboveground biomass of forest plants may thus have a profound influence on the cycling of P. The community composition of the decomposers involved in the litter decomposition may additionally depend on the lower N:P ratio of the plant material. A microcosm experiment by Güsewell & Gessner (2009), for instance, showed that the relative importance of fungi decreases while bacteria are promoted at lower litter N:P ratios. Although herbivory may also interact with the nutrient availability and uptake by plants (Anderson 2003), this and other multitrophic interactions largely remain to be studied.

4.2 Land-use effects on aboveground plant biomass

Past land use had a substantial impact on the total aboveground biomass of most forest herbs. We acknowledge that total biomass is only a rough measure of plant performance, but it may be considered an integrated estimation of overall performance related to plant interactions (e.g., competition). The observation that the species mainly responded in terms of P, and not N uptake, at least suggests that of the two nutrients P is the one that may be partly associated with the increased biomass in the post-agricultural stands, especially if we assume N to be a non-limiting nutrient at our sites (see above). In the pot experiment of Chapter 5 we found higher biomass production in response to P supply for particular species, but differences in light availability also had an effect. If land-use history affects the composition and structure of the canopy, e.g. with higher light availability in post-agricultural sites, this might also account for the observed plasticity in biomass production of *U. dioica*, *A. podagraria* and *G. urbanum* (Pigott and Taylor 1964; Elemans 2004; Chapter 5). The relative importance of light availability was, however, not explicitly determined here.

There were also clear inter-specific differences in biomass response to the former land use and those differences were partly related to colonization capacity: the fast colonizing species were able to increase their biomass more than the slow species in post-agricultural versus ancient forest. Although the relation between the colonization capacity index and the relative biomass was not particularly tight (Fig. 4.3), it showed that the slow colonizing species not only suffer from lower dispersal and recruitment rates (Verheyen et al. 2003c; Chapter 2 and 3), but additionally have a lower ability to benefit from the altered habitat quality. While *U. dioica* almost increased its biomass by a factor four in post-agricultural compared to ancient forest, the increase for *A. nemorosa*, *P. elatior* and *C. lutetiana* was smaller (maximum a factor 2.2). In this way, individuals of *U. dioica* (and *G. urbanum*) became much larger than the slow species in post-agricultural forest (e.g., the biomass of *P. elatior* individuals was on average 3.5 times lower). The strong response of *U. dioica* to former land use, and P in particular, has been found elsewhere and is expected to be a hindrance to the recruitment and growth of slow colonizing forest plants (e.g., Endels et al. 2004; De Keersmaeker et al. 2004; Chapter 3). In high nutrient environments, vigorously growing tall species such as *U. dioica* are able to project their leaves above those of neighbouring individuals, which gives them a disproportionate advantage to compete for light (Craine 2009; Hautier et al. 2009). While previous research already provided evidence that the nutrient legacies in post-agricultural forests might hamper particular recruitment stages of slow colonizing forest species (see Chapters 2 and 3), the present study shows that those species may additionally have the disadvantage of being less plastic in terms of biomass production compared to the fast colonizers. This may accentuate their low colonization abilities.

CHAPTER 5 – PLASTICITY IN RESPONSE TO PHOSPHORUS AND LIGHT AVAILABILITY IN FOUR FOREST HERBS: A POT EXPERIMENT

AFTER: BAETEN L, VANHELLEMONT M, DE FRENNE P, DE SCHRIJVER A, HERMY M, VERHEYEN K (2010) PLASTICITY IN RESPONSE TO PHOSPHORUS AND LIGHT AVAILABILITY IN FOUR FOREST HERBS. *OECOLOGIA* 163, 1021-1032.

Abstract

The differential ability of forest understorey plants to colonize secondary forests on former agricultural land is generally attributed to different rates of dispersal. After propagule arrival, however, establishing individuals still have to cope with abiotic soil legacies from former agricultural land use. We focussed on the plastic responses of forest plants to increased phosphorus availability, as phosphorus is commonly found to be persistently bioavailable in post-agricultural forest soils. In a pot experiment under field conditions, we applied three P levels to four forest herbs with contrasting colonization capacities: *Anemone nemorosa, Primula elatior, Circaea lutetiana* and *Geum urbanum*. To test interactions with light availability, half of the replicas were covered with shade cloths. After two growing seasons, we measured aboveground P uptake, vegetative and regenerative performance. We hypothesized that fast-colonizing species respond the most opportunistically to increased P availability and that a low light availability can mask the effects of P on performance. All species showed a significant increase in P uptake in the aboveground biomass. The P addition had a positive effect on the vegetative performance of two of the species, which was, however, unrelated to their colonization capacities. The regenerative performance was affected by light availability (not by P addition) and was related to the species' phenology. Forest plants can obviously benefit from the increased P availabilities in post-agricultural forests, but not all species respond in the same way. Such differential patterns of plasticity may be important in community dynamics as they affect the interactions among species.

1 Introduction

Plants can cope with spatio-temporal environmental variation by adjusting their growth and development. The ability of a given genotype to render different phenotypes subject to the environmental conditions is known as phenotypic plasticity (Bradshaw 1965; Schlichting 1986; Sultan 2000). Because a high plasticity enhances the match between phenotype and environment, an ideal species would exhibit 'infinite' plasticity, i.e., expressing optimal trait values in every environment. Yet, there are ecological limitations to phenotypic plasticity (DeWitt et al. 1998; Valladares et al. 2007), and individuals of different taxa and functional plant types may differ remarkably in the extent of their response to environmental variation (e.g., Grime et al. 1997; Valladares et al. 2006). For traits that directly relate to growth and reproduction, i.e., components of fitness, differences in plasticity between species may contribute to differences in their realized distributions. In particular, the breadth of a species' distribution reflects its ability to both maintain high performance under limited resource availability and opportunistically maximize performance under favourable conditions (cf. Sultan 2001; Griffith & Sultan 2005). The ecological consequence is that the capacity of a species to maintain populations across a range of environments contributes to its ability to thrive in alternative environments or colonize and persist in novel sites (e.g., Donohue et al. 2001).

Forests on former agricultural land are a particular example of communities in which species have to cope with alternative, novel environmental conditions. There is ample evidence of persistent (a)biotic differences between post-agricultural and ancient forest stands. The abiotic legacies of former land use are expressed in soil nutrient availability (e.g., Koerner et al. 1997; Verheyen et al. 1999; Falkengren-Grerup et al. 2006; Chapter 1 §2) and spatial heterogeneity of soil properties (Fraterrigo et al. 2005; Flinn & Marks 2007). Among other nutrients, phosphorus (P) has specifically drawn the attention. A P surplus from former fertilization was found several decades (Koerner et al. 1997; Verheyen et al. 1999; De Keersmaeker et al. 2004) or even millennia (Dambrine et al. 2007) after post-agricultural forest establishment. This higher P availability is consistently reflected in the aboveground plant biomass, as we demonstrated in Chapter 4 (cf. Falkengren-Grerup et al. 2006). In addition, Richter et al. (2006) found an extensive redistribution of P among the different pools in the soil, but remarkable little depletions in most of the bioavailable P fractions during 28 year of forest development on former fields. Therefore, the persistent, bioavailable P pool may generate new challenges for forest understorey plants that colonize post-agricultural sites. The importance of a persistent P surplus should also be considered in the face of the chronic deposition of nitrogen (N) and the impact of N on temperate forest vegetations (Gilliam 2007; Gress et al. 2007). Gress et al. (2007), for instance, showed that an increasing N availability increased the demand for P and predicted that that P limitation will become an important control over the forest vegetation dynamics. A large pool of bioavailable P might cancel out this limitation.

Forest plants greatly differ in their capaciy to colonize post-agricultural forests (Verheyen et al. 2003c). Some colonize and persist soon after the establishment of a woody canopy, but others fail to do so for centuries. Although several lines of evidence demonstrate that different rates of dispersal shape the initial colonization patterns (Honnay et al. 2002b; Verheyen et al. 2003c; Flinn & Vellend 2005), the contrasting colonization capacity among species might be accentuated if fast dispersers also have the strongest tendency to maximize performance in novel environments (cf. Chapters 3 & 4). A number of field studies found effects of previous agricultural land use on several components of performance in forest plants (e.g., Endels et al. 2004; Verheyen & Hermy 2004; Fratterigo et al. 2006; Chapter 3 & 4). Despite the difference in P availability between the ancient and post-agricultural sites, the cited studies failed to prove that P is the actual environmental factor that induced the phenotypic responses because it was confounded with several other environmental legacies from former land use (e.g., pH, nitrogen and light availability). Some experimental studies provide more insight into this matter. Pigott & Taylor (1964) and Rorison (1968) found positive responses to P addition, in terms of biomass, for seedlings of some forest plants. More recent experimental work by Hipps et al. (2005) showed similar patterns: in a glasshouse bioassay with four forest herbs growing on peat-based compost, they found species-specific effects of P addition on the P uptake and biomass of the forest herbs.

Following the recommendations to perform plasticity studies in ecologically relevant, natural settings and multifactor environments (Sultan 2000; Miner et al. 2005; Valladares et al. 2007), this study aims to extend the previous experimental work by introducing some real-world complexities. We performed a pot experiment with forest soil instead of compost, the individuals were placed under field conditions and the experiment was continued for two growing seasons. Apart from P, we also manipulated light availability. Light is not only a pivotal resource for forest understorey plant species (e.g., Neufeld & Young 2003), it may also constrain growth and mask the effect of P addition on growth and performance. A number of studies, for instance, found a positive effect of nutrient addition on the growth of forest plants, but only if light availability was not limiting (Eickmeier & Schussler 1993; Meekins & McCarthy 2000; Anderson 2003; Elemans 2004). We measured P uptake and biomass (cf. Rorison 1968; Hipps et al. 2005; Chapter 4), and also recorded a suite of plant traits related to growth and reproduction. Finally, the four forest herbs used in our study strongly differ in their capacity to colonize post-agricultural forest (Verheyen et al. 2003c; Table 5.1), which enabled us to test whether fast colonizers actually respond stronger to P availability than slow colonizers. This pot experiment is thus useful to extend the field observations of Chapter 4 by using a rigorous experimental design and more detailed measurements of plant performance. In addition, the species we used here are a subset of the species sampled in Chapter 4. We hypothesized that (i) the four forest herbs show plastic responses to P and light availability, (ii) light and P availability interactively affect the performance of individuals and (iii) the fast-colonizing species are more plastic than the slow-colonizing species.

2 Methods

2.1 Study species and experiment initiation

We used four common herbaceous forest species with a similar distribution in mesotrophic, deciduous forests in Western Europe. The four study species were specifically chosen to represent different life forms and different leaf and flowering phenologies (Table 5.1). Furthermore, the species differ in their affinity to historically continuous ‘ancient’ forest. *Anemone nemorosa* L. is the most dependent on forest continuity whereas *Geum urbanum* L. occurs frequently in post-agricultural forest as well. *Primula elatior* Hill and *Circaea lutetiana* L. have intermediate capacities to colonize post-agricultural forests (Verheyen et al. 2003c). Individuals or ramets (for geophytes) of the four study species were grown from seeds (*P. elatior, G. urbanum*) or rhizomes (*A. nemorosa*) and hibernacles (*C. lutetiana*, Verburg & Grava 1998) in small pots (0.6 litre) filled with compost soil during early spring 2007. No extra nutrients were added. We used four rhizome fragments in each pot for both *C. lutetiana* and *A. nemorosa*. Theoretically, phenotypic plasticity should be studied using genetically identical individuals that are exposed to the experimental treatments. This is, however, difficult to achieve in ecological studies, and plasticity is often measured in a more general sense (Valladares et al. 2006). We aimed to have similar levels of genetic variability across the species by collecting the seeds or ramets of each species in large populations of ancient forest sites in the same locality (Vlaams Brabant region). Furthermore, because the grown seedlings were completely randomized over the treatments, the uncontrolled intraspecific genetic differences will be captured by the unexplained variance of our statistical models.

Table 5.1 Life form, phenology and colonization capacity of the four forest herbs used in the study

Species	Family	Life form	Flowering period	Leaf phenology	Colonization capacity*
Anemone nemorosa	Ranunculaceae	Geophyte (rhizome)	Spring	Vernal	Low
Primula elatior	Primulaceae	Hemicryptophyte (rosette)	Spring	Vernal-aestival	Moderate
Circaea lutetiana	Onagraceae	Geophyte (hibernacle)	Summer	Aestival	Moderate
Geum urbanum	Rosaceae	Hemicryptophyte (rosette)	Summer	Winter green	High

* Based on data from Verheyen et al. (2003c).

In the first week of May 2007, the four species were transplanted into 2 litre pots (n = 36 per species) that were filled with soil with a sandy silt texture (mean dry weight: 1063 g soil/pot ± 17 SE, n = 10). The soil was collected in the forest 'Muizenbos' (51.197 °N, 4.566 °E), which is a mesotrophic forest characterized by a complex mosaic of stands with well-documented, contrasting land-use histories (e.g., Verheyen & Hermy 2001b, see Chapters 3 and 4). To assess whether soil factors other than P availability may also induce variation in plant performance, soil from two adjacent forest stands with a contrasting land-use history (ancient versus post-agricultural) but similar, low P concentrations was used to fill half of the pots each (n = 18 per species). The selection of the two stands was based on detailed analyses of the chemical soil conditions, notably P (De Keersmaeker et al. 2004), and land-use history (Verheyen & Hermy 2001b). The first stand has known continuous forest cover for at least 150 years whereas the second stand had been used as extensive agricultural land until it was forested in the 1950s. The dominant species in the tree layer were *Populus* ×

canadensis in the ancient forest stand and *Fraxinus excelsior* L. in the post-agricultural stand; *Corylus avellana* L. dominated the coppice layer. The soil for the pot experiment was collected by digging up the upper 5 cm of mineral soil in ten patches of 1–2 m^2 in both stands. For each of the stands, the sampled soil was profoundly mixed, and the two soil types were used to fill the 18 pots for each species. After transplantation, the species were placed under equal forest conditions for the rest of the growing season to recover from transplantation stress. To characterize initial differences among individuals, we measured the number of leaves and the length and width of the three largest leaves in each pot just after transplantation (May 2007 for the spring-flowering species, August 2007 for the summer-flowering species).

To characterize the chemical soil conditions, we analysed four samples of the soil of both stands. The soil of the ancient forest stand had an average pH-KCl of 5.2; ammonium-acetate EDTA extractable (mg/100 g dry soil) K^+ 13.1 (± 0.6 SE), Mg^{2+} 11.9 (± 0.8), Ca^{2+} 268.6 (± 3.1) and P 0.5 (± 0.07) and Kjeldahl N 235.9 (± 12.4) mg/100 g dry soil. The soil collected in the post-agricultural forest stand was characterized by a pH 4.8, K^+ 6.4 (± 0.06), Mg^{2+} 6.9 (± 0.2), Ca^{2+} 230.4 (± 8.4), P 0.6 (± 0.04) and N 241.1 (± 3.7) mg/100 g dry soil.

2.2 Experimental design and treatments

In January 2008, all pots were placed under field conditions in a homogeneous *F. excelsior* (tree layer) and *Tilia cordata* Mill. (shrub layer) dominated 1.38 ha forest stand of the ancient forest 'Aelmoeseneiebos' (50.975 °N, 3.795 °E). For each species, we placed three pots with ancient forest soil and three pots with post-agricultural forest soil into each of six fenced blocks (3 pots × 2 soil types × 6 blocks; n = 36 per species; Fig. 5.1a). To simulate two levels of light availability, half of the blocks were covered with one layer of shade cloth placed at 110 cm height (70 % light reduction, polyethylene, Fig. 5.1b) when the canopy started leafing out (third week of April). The shade cloth was removed when the leaves started falling off (third week of October). Within each block, we applied three different P levels (fully crossed with the two soil types): control, 50 mg P/pot and 210 mg P/pot. These levels were in the same order of magnitude as in the previous work by Pigott & Taylor (1964) and Hipps et al. (2005). Adjusted solutions of NaH_2PO_4 were used as P source. To account for biogeochemical P fixation in the soil, we applied 50 mL of the NaH_2PO_4 solutions at four occasions during the growing season (ten day intervals). Applications started at emergence (geophytes) or new leaf development (hemicryptophytes). The shade and P treatments were applied during the growing season of 2008 and 2009.

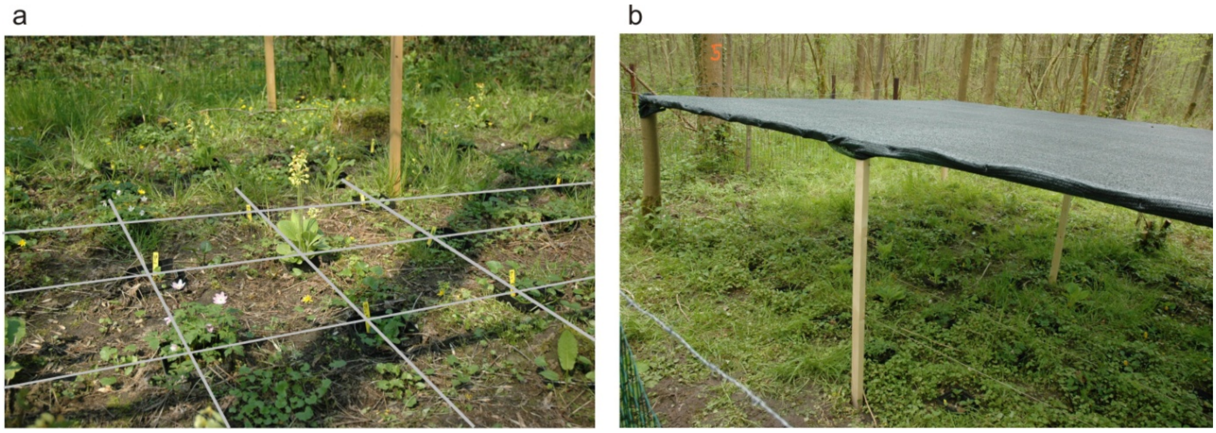


Fig. 5.1 Example of an experimental block with the pots placed at regular spacing and the species and P treatments completely randomized (a). Half of the blocks were covered with shade cloth (b) (Photographs Karen Wuyts).

2.3 Measurement of performance and P uptake

We determined plant performance by measuring a suite of species-specific traits related to growth and reproduction (cf. Endels et al. 2004; Verheyen & Hermy 2004; Chapter 3). The vegetative traits (except for *A. nemorosa*) were: total number of leaves, leaf length and total aboveground dry biomass. For ramets of *A. nemorosa*, we measured the leaf length and width, height of the leaf above the surface and biomass. The regenerative traits were: inflorescence height, number of inflorescences (not for *A. nemorosa*) and the number of flowers (*G. urbanum, P. elatior*) or the proportion of flowering ramets (*A. nemorosa, C. lutetiana*). The traits were measured on maximum three leaves and inflorescences per individual; for the geophytes, we always used the three tallest ramets. All measurements were done just following peak flowering of each species: 28 Apr 2009 (*A. nemorosa, P. elatior*), 16 Jun 2009 (*G. urbanum*) and 7 Jul 2009 (*C. lutetiana*). We also determined P in the aboveground biomass to assess whether the P added to the pots was actually bioavailable and utilized by the plants and to relate the results with data from literature and the field observations from Chapter 4. Therefore, the total aboveground biomass was harvested directly after we measured the trait values, dried for 72 hours at 70 °C and weighted (± 0.1 mg). The total P concentrations (mg P/100 g dry biomass) were measured colorimetrically by the molybdate method (Scheel 1936) following a di-acid digestion (HNO_3 65 %, $HClO_4$ 70 %, 5:1). The total aboveground P content (mg P) of individuals or ramets was calculated based on the dry biomass and the P concentration.

2.4 Data analysis

For each species, we calculated four different response variables that were evaluated against the experimental factors. First, we looked at P pools in the aboveground biomass: P concentration and total P content (concentration × total biomass) of individuals or ramets. Next, we performed a principal component analysis (PCA) on the vegetative and regenerative traits separately and extracted the first axes as measures of vegetative and regenerative performance (cf. Chapter 3). When appropriate, the trait values were log-transformed to increase the proportion of variance extracted. For each species, the first axes extracted > 61 % of either the variation in vegetative or regenerative traits, and each trait value showed a positive correlation with its associated PCA axis, i.e., there were no trade-offs between traits. The mean Pearson correlation coefficient between a trait and the first PCA axis was 0.84 (± 0.11 SD, all significant at $P < 0.0001$) for the vegetative traits and 0.90 (± 0.06 SD, all significant at $P < 0.0001$) for the regenerative traits. To account for initial differences in performance among individuals, we calculated the plant performance just following transplantation, i.e., the first axis of a PCA on the 2007 measurements (number of leaves, length and width of the longest leaf). The initial plant performance variable (INITIAL PERF) was used as a covariate in the analysis of the two response variables vegetative and regenerative performance.

The experimental design was hierarchical with the shade treatment at the block level and the P treatment at the pot level. Therefore, we applied Linear Mixed Models using the *lme* function in the *nlme* package of R version 2.9.1 (Pinheiro et al. 2009; R Development Core Team 2009) with ‘Block’ included as random effect term. We compared competing, nested models with the likelihood ratio test. The philosophy of this method is to compare the degree of belief in the competing models, rather than selecting a single model based on *P*-values (Hilborn & Mangel 1997). First, we constructed a null model with the intercepts varying randomly by block; the model parameters were maximum likelihood estimates (Model 0). The total variation in the response variable was divided among the pot level and the block level (‘intraclass correlation’). Next, we added the factor SHADE, i.e., cover by shade cloth or not, to Model 0 and performed a likelihood ratio test to evaluate whether the factor significantly improved the null model. The significance of the factor P addition was tested in the same way. Because we were mainly interested in the experimental factors SHADE and P addition, we will always report the full factorial model with both these factors and their interaction in the results (Model 1). Finally, we added the covariate INITIAL PERF (initial plant performance) or the factor SOIL (soil originating from the ancient or post-agricultural stand) to Model 1 to test whether they could additionally account for variation in the response variable. The contribution of the variable INTITIAL PERF or SOIL will only be reported if it, based on the likelihood ratio test, significantly improved Model 1. The covariate INITIAL PERF was not used in the models explaining P concentration and content. Prior to all multilevel analyses, Box-Cox transformations were applied to maximize the normality of the data.

3 Results

3.1 P concentration and total P content

Phosphorus addition had a strong effect on the P concentration and total P content in the aboveground tissue of the four forest herbs (Fig. 5.2, Table 5.2). Variability in P concentration and content was mainly related to the pot level; only for *G. urbanum*, P content also varied considerably (48 % of variation) among blocks. Imposing additional shade had a significant effect on P concentrations in the two summer-flowering herbs: a model that included the factor SHADE significantly improved the null model, based on the likelihood ratio test (Table 5.2a). In the blocks covered with a shade cloth, P concentrations tended to be higher compared to blocks without shade cloth. The P treatment was the most important factor explaining P concentrations in aboveground plant tissue of all species. The effect was most pronounced for *C. lutetiana*. The mean P concentrations of the species ranged from 260 mg P/100 g dry biomass in the control to 695 mg P/100 g dry biomass in the highest P addition treatment, i.e., a 2.7-fold increase. The full factorial models explained ≥ 63 % of the variation in P concentration for all species.

The total P content in the aboveground plant tissue showed similar responses (Table 5.2b). The P addition treatment was the main determinant for the variation in P content between individuals or ramets. The effect of SHADE was no longer significant for *C. lutetiana*, but tended to be still important for *G. urbanum*, which showed a higher total P content in blocks with just canopy shade (48 % variance at the block level of which 17 % was explained by the factor SHADE). Tissue P concentration below the shade cloths was higher, but total biomass was lower (cf. vegetative performance) compared to blocks without shade cloth, which partly levelled out the effect of SHADE on total P content. For the two geophytes *A. nemorosa* and *C. lutetiana*, introducing the factor SOIL further improved the model. The mean total P content of the two species was higher in the soil originating from the post-agricultural stand because of a higher biomass (cf. vegetative performance), not because of higher P concentrations.

Table 5.2 Effects of treatments (SHADE, P addition and SOIL) on the (a) aboveground P concentration and (b) total P content of four forest herbs tested with Linear Mixed Models. See methods for details on the model building.

	Spring-flowering		Summer-flowering	
	A. nemorosa	*P. elatior*	*G. urbanum*	*C. lutetiana*
a) P concentration				
Variance (explained)				
block level	< 0.1 %	< 0.1 %	< 0.1 %	< 0.1 %
pot level	> 99 % (63 %)	> 99 % (65 %)	> 99 % (66 %)	> 99 % (73 %)
Likelihood ratio				
L.Ratio$_{\text{SHADE}}$	0.2	< 0.1	5.0*	5.3*
L.Ratio$_{\text{P}}$	69.1***	65.3***	58.9***	62.2***
Source of variation				
SHADE	1.4	< 0.1	30.1**	53.5**
P	93.2***	99.7***	93.4***	146.6***
SHADE × P	0.4	2.6	2.8	4.8*
SOIL				6.4*
b) Total P content				
Variance (explained)				
block level	< 0.1 %	< 0.1 %	48 % (17 %)	< 0.1%
pot level	> 99 % (32 %)	> 99 % (36 %)	52 % (27 %)	> 99 % (50 %)
Likelihood ratio				
L.Ratio$_{\text{SHADE}}$	1.4	0.7	2.4	< 0.1
L.Ratio$_{\text{P}}$	8.7*	22.1***	16.9***	42.1***
Source of variation				
SHADE	1.4	1.4	2.5	0.1
P	6.6**	16.8***	10.0***	40.0***
SHADE × P	2.4	4.1*	0.7	0.2
SOIL	10.1**			4.6*

Notes: Variance = the fraction of the variation in P concentration or content at the block and pot level with the % variance explained by the model within parentheses. Likelihood ratio = test statistic and significance to test for whether SHADE (L.Ratio$_{\text{SHADE}}$) or P (L.Ratio$_{\text{P}}$) improves the null model with random intercepts (grouping factor 'Block'). Source of variation = F-values and significance for the different factors. The F-values for SHADE are tested with 4 denominator d.f., the other factors with 26 denominator d.f.; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

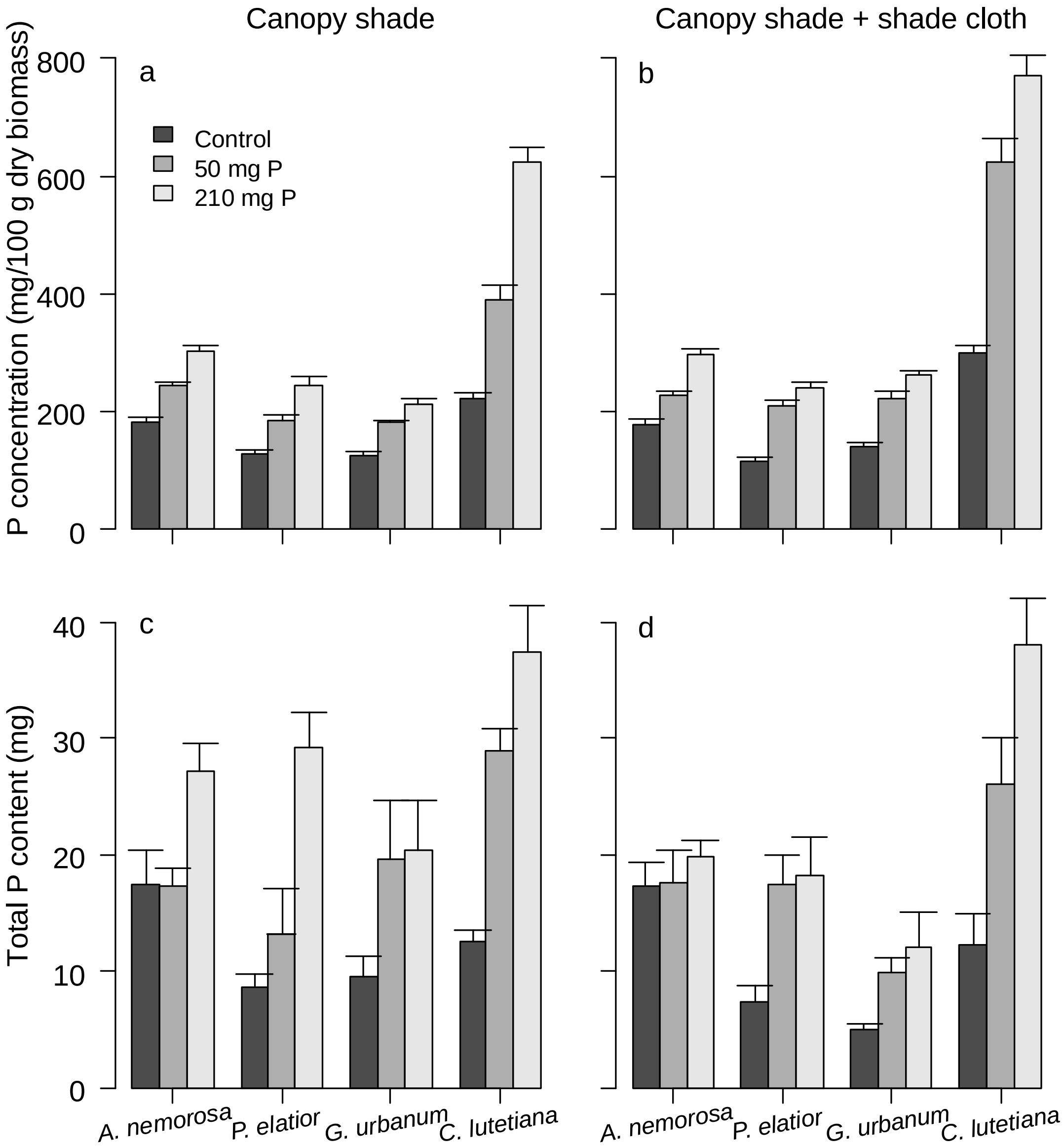


Fig. 5.2 Aboveground P concentrations (a, b) and total P content (c, d) of four forest herbs (mean ± SE) in response to P addition and SHADE treatments (n = 36 per species). The total P content of *A. nemorosa* and *C. lutetiana* was rescaled to the content in 100 and 25 ramets, respectively. Results of the multilevel models are reported in Table 5.2.

3.2 Vegetative and regenerative performance

The vegetative performance of *P. elatior* and *C. lutetiana* was significantly higher in pots with experimental P addition (significant F-value and L.Ratio$_P$); *A. nemorosa* and *G. urbanum* did not significantly react to the P treatment (Table 5.3a, Fig. 5.3). SHADE affected the vegetative performance of the two summer-flowering species: 55 % and 15 % of the variation was at the block level of which 38 % and 64 % could be explained by including the factor SHADE. The vegetative performance of the spring-flowering species was unrelated to SHADE. The regenerative performance was largely unrelated to the P addition treatment (Table 5.3b, Fig. 5.4), but SHADE did have an effect. The regenerative performance of the summer-flowering species was considerably lower in blocks covered by a shade cloth. The majority of performance variation of *G. urbanum* and *C. lutetiana* was at the block level (69 % and 72 %), and the factor SHADE accounted for 54 % and 90 % of this variation, respectively. Adding the factor SHADE significantly improved the null model (cf. L.Ratio$_{SHADE}$). Furthermore, the variation in vegetative performance was not independent from the regenerative performance. The two axes of trait variation showed a significant positive correlation for all four species ($R^2 \geq 0.47$; $P < 0.001$).

Primula elatior was the only species for which the vegetative and regenerative performance after two growing seasons was still significantly related to its performance in the first growing season (INITIAL PERF). The covariable INITIAL PERF explained 18 % and 21 % of the variation in vegetative and regenerative performance of the species in addition to a model with P and SHADE. For the other species, the initial performance differences were no longer found after two growing seasons. *Anemone nemorosa* showed a clearly different vegetative and regenerative performance in pots with soil from the different forest stands: its performance was, on average, higher in pots filled with soil from the post-agricultural stand. For *C. lutetiana*, including the factor SOIL marginally improved the models (L.Ratio $P < 0.08$), which also suggests a higher vegetative and regenerative performance in the pots with post-agricultural soil.

Table 5.3 Effects of treatments (SHADE, P addition and SOIL) and initial plant performance (INITIAL PERF) on the (a) vegetative performance (first PCA axis of vegetative traits) and (b) regenerative performance (first PCA axis of regenerative traits) of four forest herbs tested with Linear Mixed Models. See methods for details on the model building.

	Spring-flowering		Summer-flowering	
	A. nemorosa	*P. elatior*	*G. urbanum*	*C. lutetiana*
a) Vegetative performance				
Variance (explained)				
block level	12 % (< 0.1 %)	< 0.1 %	55 % (38 %)	15 % (64 %)
pot level	88 % (53 %)	> 99 % (46 %)	45 % (5 %)	85 % (26%)
Likelihood ratio				
$\text{L.Ratio}_{\text{SHADE}}$	1.1	1.3	2.5	3.6
$\text{L.Ratio}_{\text{P}}$	2.6	7.3*	0.4	8.9*
Source of variation				
SHADE	0.9	1.6	2.6	4.2
P	2.1	4.8*	0.2	4.3*
SHADE × P	1.6	1.4	0.5	< 0.1
INITIAL PERF		9.2**		
SOIL	20.0***			
b) Regenerative performance				
Variance (explained)				
block level	6 % (48 %)	< 0.1 %	69 % (54 %)	72 % (90 %)
pot level	94 % (34 %)	> 99 % (40 %)	31 % (16 %)	28 % (13 %)
Likelihood ratio				
$\text{L.Ratio}_{\text{SHADE}}$	2.8	2.7	4.3*	11.6***
$\text{L.Ratio}_{\text{P}}$	2.9	2.9	4.3	3.2
Source of variation				
SHADE	3.0	3.5	5.3	29.5**
P	1.7	1.9	2.0	1.5
SHADE × P	1.7	1.9	0.3	0.4
INITIAL PERF		7.8**		
SOIL	5.9*			

Notes: Variance = the fraction of the variation in performance at the block and pot level with the % variance explained by the model within parenthesis. Likelihood ratio = test statistic and significance to test for whether SHADE ($\text{L.Ratio}_{\text{SHADE}}$) or P ($\text{L.Ratio}_{\text{P}}$) improves the null model with random intercepts (grouping factor 'block'). Source of variation = F-values and significance for the different factors or covariate. The F-values for SHADE are tested with 4 denominator d.f., the other factors with 26 denominator d.f.; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

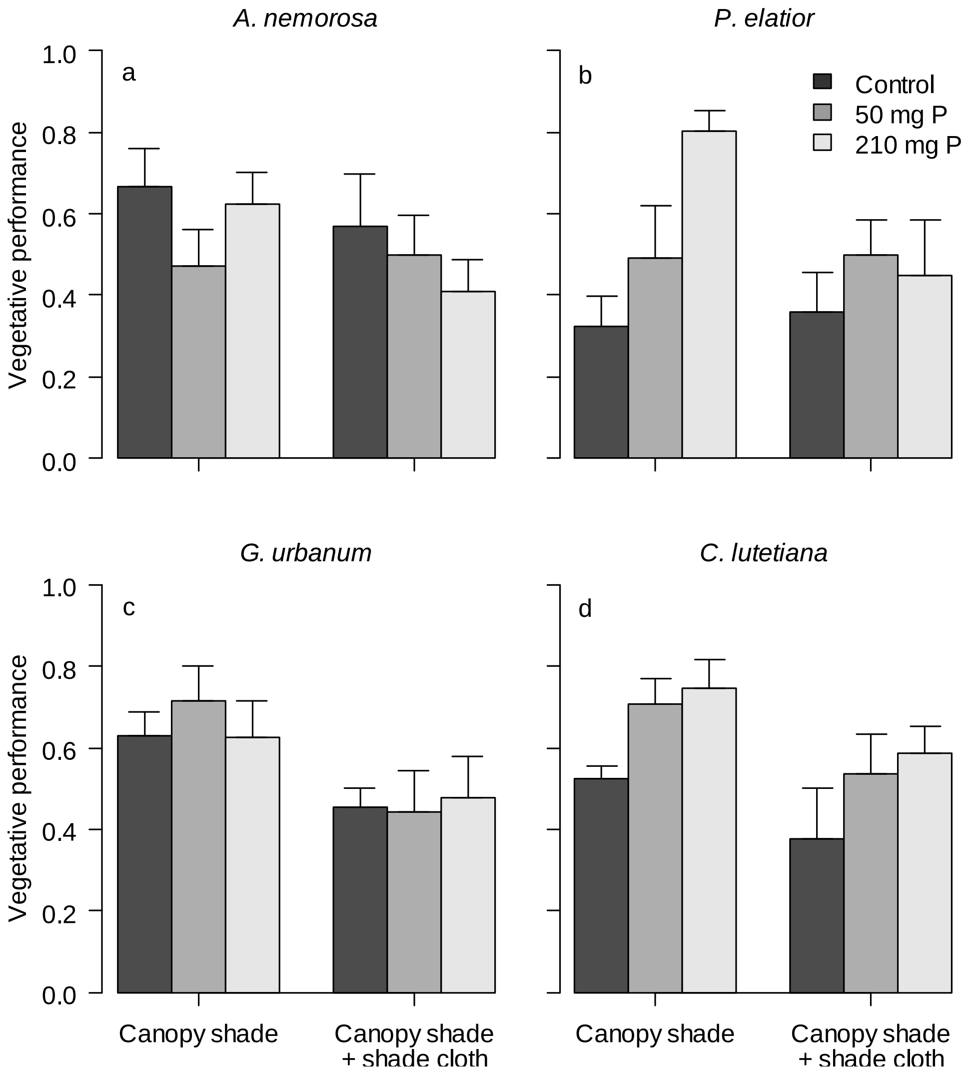


Fig. 5.3 Effects of P addition and SHADE on the vegetative performance of four forest herbs (mean ± SE, n = 36 per species). Vegetative performance is the normalized first component of a PCA analysis on vegetative traits. Results of the multilevel models are reported in Table 5.3a.

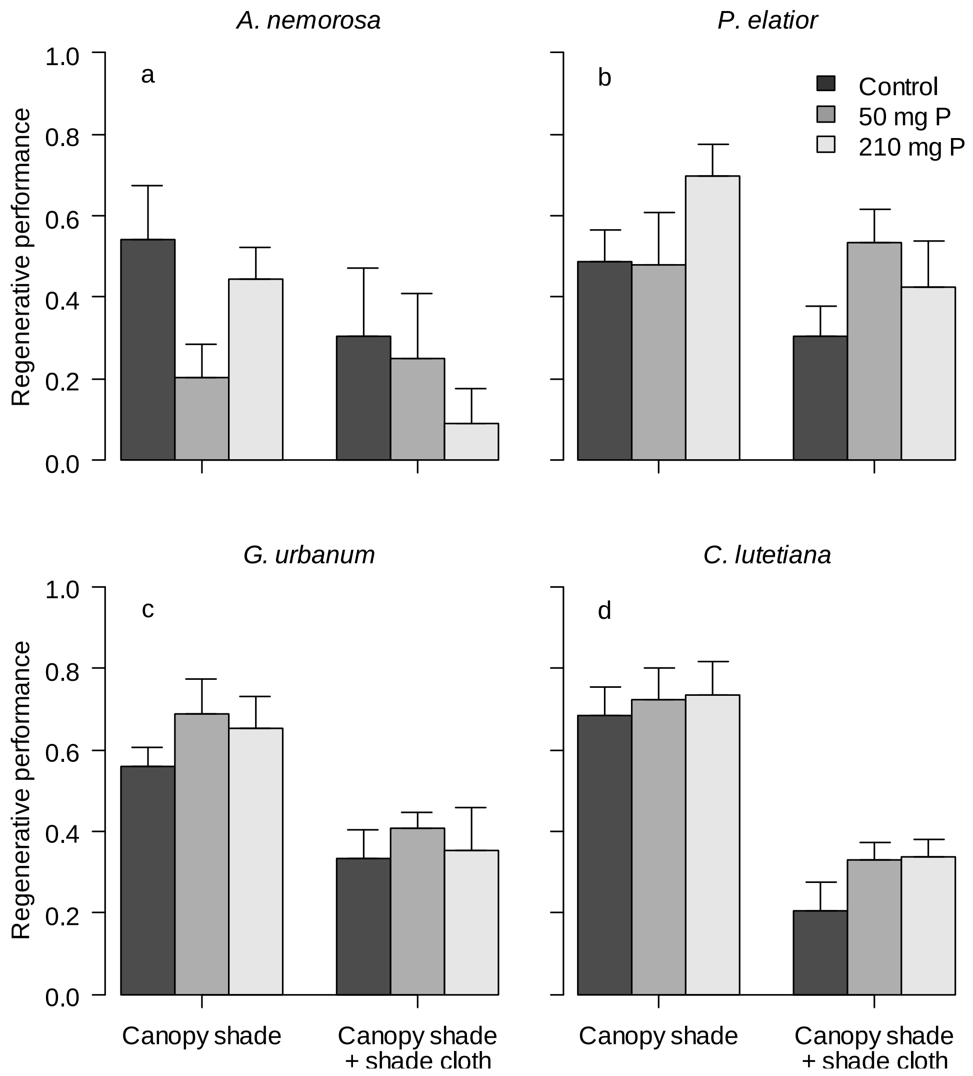


Fig. 5.4 Effects of P addition and SHADE on the regenerative performance of four forest herbs (mean ± SE, n = 36 per species). Regenerative performance is the normalized first component of a PCA analysis on regenerative traits. Results of the multilevel models are reported in Table 5.3b.

4 Discussion

The majority of research on the relationship between herb layer communities and land-use history has produced persuasive evidence that differences in dispersal ability initially control species distributions and abundances in post-agricultural forests (reviewed in Flinn & Vellend 2005; Hermy & Verheyen 2007). After propagule arrival, however, individuals still need to pass through several life-stages before they establish as reproductive adults that contribute to the maintenance of a population. The number of studies that explore how the (a)biotic legacies of former agricultural practices influence the establishment, performance and persistence of forest understorey plant species is gradually increasing (e.g., Verheyen &

Hermy 2004; Vellend 2005; Fraterrigo et al. 2006; Flinn 2007; Chapters 2, 3 and 4). In the present study we tested whether the persistent P surplus in post-agricultural forests can cause differences in performance among forest plants and how light availability additionally affects the responses.

4.1 P uptake

The aboveground P concentrations of the four forest herbs in this study all increased in response to the two levels of P addition (Table 5.2, Fig. 5.2). The species thus effectively utilized the P surplus and realized a higher P uptake, which indicates physiological plasticity (*sensu* Bradshaw 1965). The increase was typically not proportional to the supply, and in the P treatments, each species maintained concentrations that were well above those of the control, but still below the toxic levels of 1 g P/100 g dry biomass suggested by Marschner (1995). *Circaea lutetiana* turned out to be the most plastic as it concentrated 2.7 times more P in its tissue in the highest P treatment compared to the control. These patterns closely match the experimental results obtained by Rorison (1968, hydroculture) and Hipps et al. (2005, peat-based compost), which partly generalizes their results to more natural settings (i.e., mineral soil, two growing seasons, field conditions). The two summer-flowering species concentrated P more under the shade cloths, but as this was mainly the consequence of a lower biomass, the total aboveground P content did not significantly differ between shaded and unshaded blocks. Anderson (2003) similarly reports higher tissue P concentrations because of lower biomass in shaded plants of the spring ephemeral *Claytonia virginica* L. compared to unshaded plants. The ranges of tissue concentrations in our experiment are also comparable to, or even lower than, the concentrations found in field studies that sampled populations in post-agricultural forest sites in Sweden and Belgium (Falkengren-Grerup et al. 2006; Chapter 3: *P. elatior* 293 mg P/100 g dry biomass and *G. urbanum* 426 mg P/100 g dry biomass; Chapter 4). The applied P levels in our pot experiment thus probably resemble the prevailing pools of bioavailable P under field conditions. Total P content, unlike P concentration, accounts for differences in biomass among individuals and integrates uptake throughout the growing season. Accounting for biomass introduced a considerable proportion of unexplained variation (Table 5.2b), but the aboveground total P content was still correlated with an increase in P supply. Next to the effects on individual plant performance, which will be discussed below, the accumulation of P into the herbaceous layer might have important consequences for the functioning of post-agricultural forest ecosystems. Because of the rapid decomposition of herbaceous litter, which typically decomposes more than twice as fast as tree leaf litter, the herb layer significantly contributes to the efficiency of the nutrient cycling in forests (Muller 2003; Gilliam 2007). The rapid turnover of P-rich herbaceous litter is likely to be an important process for community dynamics as it directly feeds back upon P availability to plants.

4.2 Vegetative performance

An important objective of this study was to determine whether P availability affects the performance of forest plants and, if so, whether the effects are related to the capacity of the species to colonize post-agricultural sites (first and third hypothesis). The vegetative performance of two species (*P. elatior, C. lutetiana*) was positively related to P availability whereas the performance of the two other species was not (*G. urbanum*) or even slightly negatively (*A. nemorosa*) related to P supply (Table 5.3a, Fig. 5.3). Increased P availability as a legacy from former land use may thus directly affect plant performance, but not all species respond in the same way. If different patterns in plasticity among species contribute to differences in their capacity to colonize and persist in post-agricultural forest, i.e., shaping their realized distribution, fast colonizers should respond the most opportunistically to increased P availability (third hypothesis). However, our study does not provide unequivocal evidence for this idea. The two species that increased performance have moderate colonization capacities, while the fast colonizing *G. urbanum* was unaffected.

These patterns seem to be partly inconsistent with earlier observations in the eight-year introduction experiment of Chapter 3 that used three corresponding forest herbs. In this introduction experiment, *G. urbanum* actually did perform better and persisted in P-enriched post-agricultural sites (cf. Endels et al. 2004), so there must be environmental variables other than P that additionally affect performance (e.g., light availability). On the other hand, *P. elatior* indeed had the intrinsic ability to show higher performance in P-enriched post-agricultural sites (Verheyen & Hermy 2004), but due to indirect effects such as low longevity and competition with opportunistically growing species taking advantage of the high P availability (e.g., *Urtica dioica* L.: Pigott & Taylor 1964; Hipps et al. 2005; Taylor 2009), the species could not maintain viable populations (Chapter 3). Similar indirect effects of P availability, via competition, on the growth of forest plants were also found by Hipps et al. (2005). Finally, the present pot experiment showed that the spring geophyte *A. nemorosa* was unaffected or even slightly negatively affected by P addition (cf. Verheyen & Hermy 2004; Hipps et al. 2005; Chapter 3 and 4), but the use of soil from two adjacent forest stands introduced a significant amount of variation in both the vegetative and regenerative performance of the species. The two soil types slightly differed in exchangeable cations (mainly K^+ and Mg^{2+}), but other drivers related to land-use history such as the composition of soil biota may equally well account for this effect. Although we must acknowledge that land-use history (post-agricultural versus ancient forest soil) was not truly replicated, it is a remarkable result that *A. nemorosa* performed better in the post-agricultural soil, which was not P enriched, but tended to be negatively affected by additional P supply. Further study is required to unravel the complex effects – which seem to be beneficial as well as detrimental – of former land-use on the growth of similar forest understorey species.

In Chapter 4, we showed that six out of seven forest herbs increased their biomass in post-agricultural versus ancient forest. *Anemone nemorosa* rather showed the opposite response. It was suggested that an increased P availability may partly account for the effect of former land use on understorey plant performance. The experimental results presented here clearly underpin this suggestion for *P. elatior, C. lutetiana* and *A. nemorosa* (rather negative effect), but not for *G. urbanum*, which seems to respond to other legacies of former land use (see above). This further emphasises the benefit of combining both field and experimental data to unravel the impact of former land use on understorey plant species.

4.3 Regenerative performance

While the regenerative performance was largely unaffected by the P treatment, light availability had a major impact on the regenerative performance of the two summer-flowering herbs (Table 5.3b, Fig. 5.4). The number and height of inflorescences and the number of flowers (*G. urbanum*) or flowering frequency (*C. lutetiana*) were reduced in experimental blocks that were covered with a shade cloth. In an allocation study, Verburg & Grava (1998) also found positive effects of light availability on the reproductive effort of *C. lutetiana*. They showed that fruit production was size dependent, which is consistent with the positive relationship we found between vegetative and regenerative performance of the species. Similarly, in an ecophysiological study, Pons (1977) showed that *G. urbanum* performed better in terms of biomass in high light compared to low light conditions. The dominant effect of light availability on the performance of *G. urbanum* and the lack of response to P addition at least suggests that the higher performance of the species in post-agricultural compared to ancient forest sites (Endels et al. 2004; Verheyen & Hermy 2004; Chapter 3) is partly the result of differences in light conditions. The spring-flowering species avoid canopy shade through phenological escape and were not affected by the shade treatment. Since we only measured a suite of morphological traits to determine the extent of regenerative performance, it remains to be studied how other components of reproductive output such as the number of seeds, seed mass and germination are affected by former land use. This topic will be elaborated in Chapter 6. Based on previous experimental work on the effects of nutrient and light availability on the growth of forest plants (e.g., Meekins & McCarthy 2000; Elemans 2004), we expected interactive effects of P and SHADE on P uptake and plant performance (second hypothesis). Experiments with the spring ephemeral *C. virginica* in a deciduous forest in Tennessee, for instance, showed that NPK fertilization increased the vegetative and reproductive biomass of plants growing at ambient sunlight more than for plants growing under experimental shade treatments (Eickmeier & Schlusser 1993; Anderson 2003). It was suggested that shaded plants have a lower demand for nutrients because low light constrains their growth so that the plants may not be able to respond to increased nutrient availability at lower light levels. In our study, the interactive effects of P and SHADE on the aboveground P concentration (*C. lutetiana*) and total P content (*P. elatior*) also indicated that shading may partly mask the response of forest plants to higher nutrient availability. For the performance measures, we did not find significant interaction terms in the multilevel models, but synergistic effects of P and SHADE on the overall plant performance did emerge. For *C. lutetiana*, vegetative traits were positively related to the addition of P whereas light mainly affected regenerative traits. So, the overall performance in this species obviously depended on the availability of both resources.

We used four herb species with differing combinations of life form and phenology to uncover response patterns related to these life-history characteristics. Although light differentially affected the spring and summer-flowering species, the effect of P availability was unrelated to life form and phenology. This result suggests that responses to P availability are largely species specific, or related to other life-history attributes, which turns it difficult to make general predictions about other species. Similarly, Fratterigo et al. (2006) found species-specific effects of land-use history on the growth and performance of twelve forest plants in southern Appalachian forests. Because it remains unclear which traits or trait combinations related to performance are promoted by former agricultural land use, and more specifically P availability, it is difficult to predict how P exactly contributes to the structuring of herbaceous communities in post-agricultural forest. The potential effects of P availability on forest communities should also be considered in relation to other key resources. A persistent P surplus might, for instance, provide the increasing demand for P that is expected at high levels of chronic N deposition (Gress et al. 2007). This would clear the way for highly productive forest vegetations in which forest plants that have the ability to benefit from P (e.g., *P. elatior, C. lutetiana*) are still competitively excluded.

To conclude, the persistently higher P availability in post-agricultural forests may have positive, species-specific effects on the vegetative performance of forest plants (cf. first hypothesis; Rorison 1968; Hipps et al. 2005). The regenerative performance, on the other hand, was mostly related to light availability. It is important to notice, however, that pot experiments only determine the potential plastic response of individuals and ignore complex community interactions. Comparing our results with extensive field observations (cf. Chapter 3) at least suggests that indirect effects such as competition may cancel, if not reverse, the effects of P availability on population persistence in herbaceous communities. *Primula elatior* proved to be an informative case as it benefits from P addition, but, simultaneously, suffers from competition with P-limited competitors in field conditions (Chapter 3). Addressing how differences in the plastic response among forest plants alter the magnitude of interactions between species (trait-mediated interactions *sensu* Callaway et al. 2003) and structure forest plant communities is an important direction for future research. Furthermore, land-use history not only influences the distribution and abundance of forest plant populations, it also impacts their genetic diversity. Vellend (2004), for instance, found reduced genetic diversity and genetic divergence (drift) in *Trillium grandiflorum* (Michx.) Salisb. populations in secondary forests via land-use effects on effective population size. Jacquemyn et al. (2009) showed a rapid decline in genetic diversity in a founding population of *P. elatior* colonizing recent forest stands. We therefore argue to perform similar experiments such as the present on individuals from different populations (e.g., populations in ancient versus post-agricultural forest) and genetically distinct groups to explore how genetic variability controls the plasticity of forest plants and to gain further insights into the complex effects of land-use history on forest plant populations.

CHAPTER 6 – LAND-USE LEGACIES CAN AFFECT THE PRODUCTION OF GERMINABLE SEEDS IN *PRIMULA ELATIOR* AND *GEUM URBANUM*

AFTER: BAETEN L, VANHELLEMONT M, DE FRENNE P, HERMY M, VERHEYEN K. THE PHOSPHORUS LEGACY OF FORMER AGRICULTURAL LAND USE CAN AFFECT THE PRODUCTION OF GERMINABLE SEEDS IN FOREST HERBS. *ECOSCIENCE*, IN PRESS. DOI 10.2980/17-4-3372

Abstract

Land-use history can have large effects on the different life stages and demography of forest plant species. Here we studied how the legacies of former land use in post-agricultural forests, and increased phosphorus availability in particular, may alter the germinability and seed quantity in populations of the forest herbs *Primula elatior* and *Geum urbanum*. We collected seeds in experimental populations of *P. elatior* and *G. urbanum* established in post-agricultural and ancient forest stands ten years ago and determined the number of seeds per fruit and seed germinability. The effect of P availability on the production of germinable seeds was tested in a pot experiment with three P levels. Former land use had an impact on the mean germination percentage: seed germinability tended to be higher in post-agricultural compared to ancient forest sites. For *G. urbanum*, the number of seeds per fruit was also higher in post-agricultural forest. Whereas P availability had no effect on *G. urbanum* seed quantity and germinability, the germination percentage of *P. elatior* seeds increased significantly with P supply. Whereas previous studies showed that former agricultural land use can have detrimental effects on particular life stages of forest plants (e.g., reduced juvenile or adult survival), the production of germinable seeds might rather benefit from it. The environmental legacies of former land use thus affect the various life stages of a plant differently, which results in complex effects of land-use history on the demography of forest plants.

1 Introduction

The patch occupancy and local abundance of plant populations is generally constrained by the amount of germinable seeds arriving at potential recruitment sites, termed seed limitation (e.g., Eriksson & Ehrlén 1992; Turnbull et al. 2000; Clark et al. 2007). Seed limitation basically arises from (1) a restricted production of germinable seeds (source limitation) and (2) a restricted ability of seeds to reach the recruitment sites (dispersal limitation) (Clark et al. 1998; Clark et al. 2007). Seed limitation may be particularly important for the recovery of plant populations following an extensive disturbance that removed virtually all individuals from a habitat patch. First, plants have to recolonize from nearby remnant populations through dispersal, and second, the local seed production of successfully established individuals will primarily determine the local abundance of the species.

Forests established on former agricultural land have served as a particularly useful study system to determine the importance of seed limitation after a disturbance. A large number of studies have shown that dispersal limitation initially hampers the successful colonization of forest understorey plant species into post-agricultural forests (reviewed in Hermy et al. 1999; Flinn & Vellend 2005; Hermy & Verheyen 2007; Chapter 1 §3.1), and the isolation of unoccupied sites through strong habitat fragmentation makes seed dispersal even more difficult (e.g., Jacquemyn et al. 2001; Honnay et al. 2002b). Yet, occasional long-distance dispersal events, for instance through deer herbivory on seed-bearing adults, may ultimately result in the establishment of small founding populations in post-agricultural forests (Vellend et al. 2003). Once these populations have established (cf. Chapters 2 and 3), their maintenance and spread may primarily rely upon the seed production of the established adults (source limitation). Vellend (2004) and Jacquemyn et al. (2009) for instance studied the population genetics of *Trillium grandiflorum* (Michx.) Salisb. and *Primula elatior* Hill in post-agricultural forests and showed that there was low gene flow from outside, i.e., the majority of young individuals were offspring of parent plants within the established populations. Determining how former land use affects the production of germinable seeds (i.e., the number of seeds and their germinability) may therefore significantly contribute to our understanding of the demography in the colonizing populations.

In the small founding populations, some biotic limitation to seed production may arise, e.g., through reduced mate availability and altered pollinator interactions (e.g., Jacquemyn et al. 2002; Honnay et al. 2005). In addition, the former land use may affect the seed production via its effect on the local abiotic soil conditions. The availability of phosphorus (P), among other nutrients, is considered one of the most persistent abiotic legacies of agricultural fertilization. A P surplus was found several decades to even millennia after agricultural abandonment (e.g., Koerner et al. 1997; Verheyen et al. 1999; Dupouey et al. 2002; Richter et al. 2006) and causes increased aboveground P uptake in forest plants (Dupouey et al. 2002; Falkengren-Grerup et al. 2006; Chapters 3–5). Furthermore, the growth and performance of adult forest plants may benefit from the increased levels of bioavailable P (Verheyen & Hermy 2004; Hipps et al. 2005; Fraterrigo et al. 2006; Chapters 3–5). Nevertheless, it remains largely unexplored how the increased P uptake and increased performance of mother plants relates to the production of germinable seeds (but see Endels et al. 2004). Maternal effects of the soil nutrient availability on the reproductive output may come from a positive relation between the seed mineral composition and germinability and between the plant performance and seed number or germinability (e.g., Roach & Wulff, 1987; Allison, 2002).

In the present Chapter, we aimed to determine the effect of former land use, and P supply in particular, on the number of seeds and their germinability in two forest herbs (*P. elatior* and *Geum urbanum* L.). The two species were specifically selected because they showed clearly diverging trends in population growth at ancient versus post-agricultural forest sites in Chapter 3. We sampled seeds from the experimental populations in Muizenbos forest (Chapter 3) to determine whether different land-use effects on the production of germinable seeds partly account for the opposite trends in population growth, i.e., *G. urbanum* successfully expanding and *P. elatior* decreasing. In addition, we sampled seeds from both species in the pot experiment of Chapter 5 to explore whether P availability is the factor that may underlie a land-use effect on the seed production and germination percentage. We hypothesised that (i) former agriculture has an effect on the number of seeds and their germinability; (ii) the increased P availability in post-agricultural versus ancient sites partly accounts for the land-use effect and (iii) the successfully expanding *G. urbanum* is positively affected, while *P. elatior* is not.

2 Methods

Here we briefly describe the two experiments that we used to sample the seeds of *P. elatior* and *G. urbanum*. For details on the study designs of the experimental introduction we refer to Chapter 3. The pot experiment is extensively described in Chapter 5. The autecology of the two study species, including details on their seed ecology can be found in the Methods of Chapter 3.

2.1 Seed collection: experimentally established populations and pot experiment

In 1999, experimental populations of *P. elatior* and *G. urbanum* were established in two ancient forest sites (forest for more than 145 year based on the oldest land-use maps) and four recent afforestations (*c.* 15 year old) on former heavily fertilized grasslands in the forest 'Muizenbos', central Belgium (51.197 °N, 4.566 °E). The soils were Quarternary niveo-eolian sandy loam and silt loam deposits supporting typical mesotrophic plant communities. The canopy in ancient and post-agricultural sites was similar and was dominated by *Populus* × *canadensis* in the tree layer and *Alnus glutinosa* (L.) Gaertn. and *Fraxinus excelsior* L. in the subcanopy. In each site, the two forest herbs were introduced into six 0.5 m × 0.5 m plots by sowing seeds and into 6 similar plots by planting adults. Seed densities were 400 seeds.m^{-2} and the number of adults per plot was four (*P. elatior*) or two (*G. urbanum*). Because the populations in both ancient and post-agricultural forest were established at the same time using a similar number of seeds and adults, the effects of land use are largely isolated from potential population size effects. We collected whole fruits of *P. elatior* (3–5 fruits) and *G. urbanum* (2–3 fruits) from 5 adults in each of the established populations in the two ancient sites and three of the post-agricultural sites. Fruits were collected in the first week of July 2009, i.e., when the two species are also dispersed naturally.

In the pot experiment, individuals of *P. elatior* and *G. urbanum* were planted in 2 litre pots filled with loamy forest soil in May 2007 (n = 18 per species). The soil (upper 5 cm of the mineral soil) was collected in the forest 'Muizenbos', i.e., the forest in which the experimental populations were established. The sampled individuals in the experimental populations and the pot experiment were thus grown on the same soil type. As part of the pot experiment in Chapter 5, the soil was collected in one ancient and one post-agricultural forest site with equal, low soil P concentrations (ancient: 0.5 [± 0.07 SE] mg P/100 g; post-agricultural: 0.6 [± 0.04 SE] mg P/100g) to fill half of the pots each. This 'soil type' treatment was introduced to account for land-use effects other than P on the performance of forest herbs. Because soil type had no effect on the adult performances of *P. elatior* and *G. urbanum* (Chapter 5) and because the treatment did not improve the statistical models of the present study (data not shown), the effect of soil type is not further considered here. After planting the species, all the pots were placed in a homogeneous *F. excelsior* (tree layer) and *Tilia cordata* Mill. (shrub layer) forest stand of the forest 'Aelmoeseneie' (50.975 °N, 3.795 °E) in January 2008. We applied each of three P levels on one third of the pots during the growing seasons 2008 and 2009: control, 50 mg P/pot and 210 mg P/pot as adjusted NaH_2PO_4 solutions. The aboveground tissue P concentrations at the highest P addition level were comparable to the values found in post-agricultural forest, so the P levels in the pot experiment probably resemble the prevailing pools of bioavailable P under field conditions. Whole fruits of each *P. elatior* (8–12 fruits) and *G. urbanum* individual (3–6 fruits) were also collected during the first week of July 2009.

2.2 Germination tests and determination of seed mass and seed number

After seed collection, the seeds were air dried at room temperature for four days. Then, we randomly chose three times 30–50 seeds of each adult individual, depending on the number of available seeds, and determined the mean seed mass of these subsamples to the nearest 0.1 mg. The total mass of the remaining seeds was also determined. The mean number of seeds per fruit was calculated as the total seed mass, i.e., the mass of the three subsamples and remaining seeds, divided by the mean seed mass and the number of sampled fruits. Next, each of the 30–50 seeds subsamples was distributed in a petri dish lined with moist filter paper (Macherey–Nagel 440B) during the week following seed collection. The dishes were immediately placed in warm incubation at 20 °C (12 h) and 10 °C (12 h) temperature regime using a Sanyo MLR-351 incubator. The filter papers were moisturized every week with distilled water. The number of germinated seeds of *G. urbanum* was recorded weekly (Fig. 6.1). We stopped counting after six weeks when germination had virtually ceased in all petri dishes (cf. Graae et al. 2009). After six weeks, the seeds of *P. elatior* were placed in cold stratification at 2 °C for 16 weeks. Then, the petri dishes were placed in warm incubation as above. We recorded the number of germinated seeds every two weeks (germination started after 13 weeks in cold stratification) until germination ceased in all petri dishes after 30 weeks.

2.3 Data analysis

We used Generalized Linear Mixed Models (GLMMs) to test for significant differences in the mean number of seeds per fruit, mean seed mass or germination percentage between ancient and post-agricultural forest and the different P treatment levels. As the germination percentage was calculated as the proportion of germinated seeds versus the total number of seeds in a petri dish, i.e., proportional data, we used binomial errors and logit link function. For the mean number of seeds per fruit and mean seed mass we used normal errors. The test statistics for the models with binomial errors and normal errors were z-values and t-values, respectively. As land-use history varied at the stand level and seeds from each adult individual were distributed over three petri dishes, we included 'Stand' and 'Adult individual' nested within stand as random effect terms for the analyses of the experimental population data. Likewise, 'Adult individual' was included as random effect term in the models analyzing the pot experiment data. The models were fitted with the *lmer* function in the *lme4* package (germination percentage) and the *lme* function in the *nlme* package (number of seeds per fruit, mean seed mass) in R 2.9.1 (Pinheiro et al. 2009; R Development Core Team 2009; Bates & Maechler 2010).

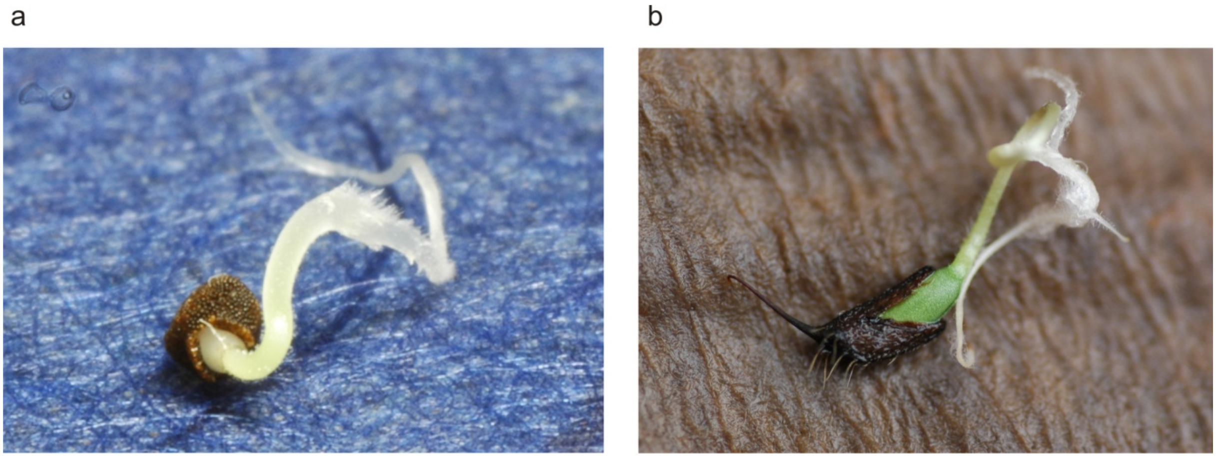


Fig. 6.1 Germinated seed of *Primula elatior* (a) and *Geum urbanum* (b) (Photograph b: Johan Eeckhout).

3 RESULTS

The germination percentage of *P. elatior* and *G. urbanum* seeds was positively related to the mean seed mass, but there were no significant differences between ancient and post-agricultural stands (Table 6.1). The lack of significance for the factor Land use was partly caused by the low level of replication (two ancient and three post-agricultural stands): a statistical model without the random effect 'Stand', i.e., a model assuming replication of land use at the individual level, indicated a significantly higher germination percentage in post-agricultural stands ($P < 0.03$ for both species; Fig. 6.2a). Furthermore, the mean number of seeds per fruit of *G. urbanum* was significantly higher in post-agricultural (104.1 ± 6.2 SE) compared to ancient forest stands (77.2 ± 4.6 SE). The overall mean number of seeds per fruit of *P. elatior* was 34.0 (± 1.8 SE), and was not affected by land-use history. Mean seed mass did not significantly ($P > 0.1$) differ between ancient and post-agricultural forest for both species.

Table 6.1 Effect of land-use history on (a) the mean number of seeds per fruit and (b) the germination percentage in two forest herbs. Seeds were collected from five adult individuals in each of two ancient and three post-agricultural forest stands (n = 25). For the germination percentage, we included the mean air dry seed mass as covariate. See methods for details on the model building.

Source	*Primula elatior*			*Geum urbanum*		
	Effect	Z/t	*P*	Effect	Z/t	*P*
a) Number of seeds						
Land use		0.173	0.873	+	2.786	0.069
b) Germination percentage						
Land use		1.091	0.275		1.586	0.113
Seed mass	+	2.459	0.014	+	3.982	< 0.001

Notes: Significances were tested with Generalized Linear Mixed Models including 'Stand' and 'Adult individual' nested within stand as random effect terms. Effects are reported if $P < 0.1$; a positive effect of 'Land use' indicates a higher value in post-agricultural versus ancient forest.

The germination percentage of seeds in the pot experiment was also positively related to the mean seed mass for the two forest herbs (Table 6.2). Whereas the germinability of *G. urbanum* seeds was unaffected by the P treatment, *P. elatior* seed germination increased significantly with P availability (Fig. 6.2b). The mean number of seeds per fruit was unaffected by the P treatments. Overall mean numbers of seeds per fruit were 40.3 (± 2.5 SE) and 72.1 (± 5.4 SE) for *P. elatior* and *G. urbanum*, respectively. Furthermore, the P treatment did not significantly ($P > 0.1$) affect the mean seed mass for both species.

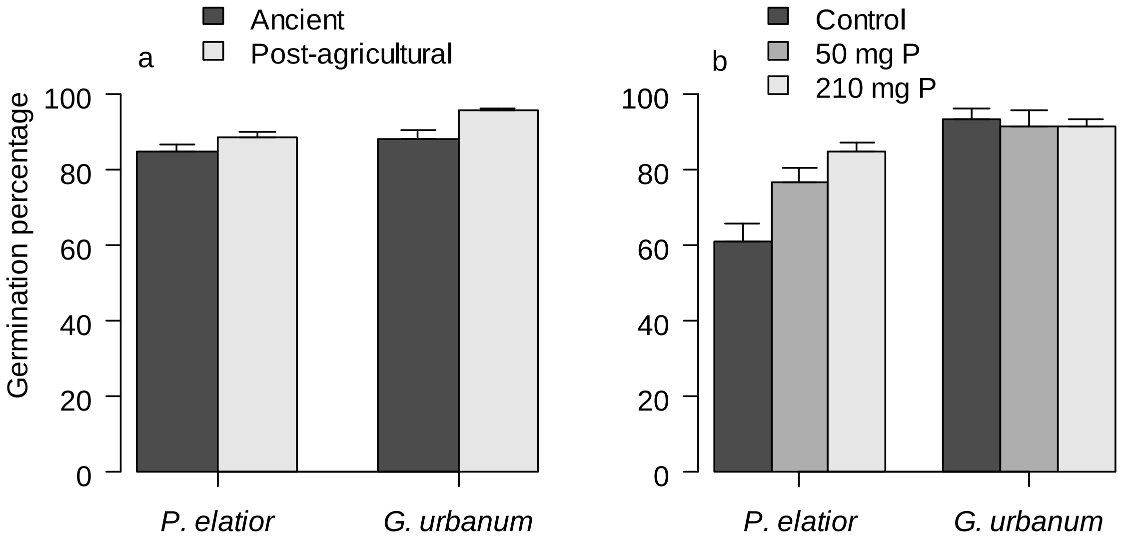


Fig. 6.2 Germination percentage of *Primula elatior* and *Geum urbanum* seeds (mean ± SE) collected from adults in (a) experimentally established populations in ancient and post-agricultural forest (n = 25 adults per species) and (b) a pot experiment with 3 levels of P addition (n = 18 adults per species).

Table 6.2 Effect of P availability on (a) the mean number of seeds per fruit and (b) the germination percentage in two forest herbs. Seeds were collected from six adult individuals in each of three P addition treatments: control, 50 mg P and 210 mg P (n = 18). For the germination percentage, we included the mean air dry seed mass as covariate. See methods for details on the model building.

Source	*Primula elatior*			*Geum urbanum*		
	Effect	Z/t	*P*	Effect	Z/t	*P*
a) Number of seeds						
50 mg P		0.005	0.996		0.562	0.594
210 mg P		-0.309	0.763		1.200	0.275
b) Germination percentage						
50 mg P		1.048	0.295		0.278	0.781
210 mg P	+	2.127	0.033		-0.625	0.532
Seed mass	+	4.260	< 0.001	+	5.568	< 0.001

Notes: Significances were tested with Generalized Linear Mixed Models including ‘Adult individual’ as random effect term. Effects are reported if $P < 0.1$; the effects of 50 and 210 mg P were tested against the control treatment, i.e., a positive effect indicates a higher response value compared to the control.

4 DISCUSSION

The colonization of forest plant populations following an intensive disturbance such as land-use change may be impeded at the different life stages of an individual. The general view is that of a two-stage colonization trajectory in which low seed availability may be followed by restricted recruitment from the seedling into the adult stage (Flinn & Vellend 2005; Hermy & Verheyen 2007; Chapters 2 & 3). Previous research on the low seed availability has almost exclusively looked at the importance of dispersal limitation, mainly by relating the distributional patterns of forest understorey plant species to the spatial and temporal isolation from source habitats (e.g., Brunet & von Oheimb 1998; Verheyen & Hermy 2001b). The effect of former land use on the production of germinable seeds in a founding population, which forms a significant component of the local seed availability (source limitation), has received virtually no attention (e.g., Endels et al. 2004).

4.1 Experimental populations: ancient vs. post-agricultural forest

We used experimentally introduced populations of two forest herbs (*P. elatior* and *G. urbanum*) to explore this research gap. The long-term monitoring of the experimental *P. elatior* populations in Chapter 3 showed that the species fails to produce new recruits in post-agricultural forest and may not persist at those sites. Therefore, we may expect that the production of germinable seeds (number and germination percentage) in *P. elatior* is reduced in post-agricultural versus ancient forest sites. Our data do not support this expectation: germination percentage was not significantly affected by land-use history and even tended to be higher in post-agricultural forest (Table 6.1, Fig. 6.2a). The low recruitment into the adult life stage thus appears to be mainly caused by limitations in the post-germination stages. In a life table response analysis, Jacquemyn & Brys (2008) for instance found evidence for the negative contribution of low juvenile survival and of low seedling and juvenile growth to population growth rates in *P. elatior*. Likewise, Endels et al. (2004) found in an observational study no differences in the germination percentage of *P. elatior* seeds between ancient and post-agricultural forest and attributed the low adult densities in the latter habitat type to reduced seedling and juvenile survival. The high interspecific competition with species from the resident community that profit from the fertilization legacies in post-agricultural forests, especially P availability, is often cited to account for the low survival of the seedlings and juveniles (Hermy et al. 1999; Endels et al. 2004; Hipps et al. 2005; Jacquemyn & Brys 2008; Chapters 2 and 3).

While the germination percentage of *G. urbanum* did not differ between the ancient and post-agricultural populations, the number of seeds benefitted from the land-use legacies (Table 6.1). This result corroborates the patterns in Endels et al. (2004): the germinability of *G. urbanum* was equal and the mean number of seeds per fruit was higher in forests established after 1775 compared to ancient forest stands. Flinn (2007) similarly found positive effects of former agricultural land use on the probability to produce spores and the number of spores in the two ferns *Dryoptheris carthusiana* (Vill.) H.P. Fuchs and *Polystichum acrostichoides* (Michx.) Schott. Previous monitoring already demonstrated that the adults of *G. urbanum* in the experimental populations had higher generative performance in post-agricultural sites (number and height of inflorescences, number of flowers), while land-use history did not affect the adult performance of *P. elatior* after eight growing seasons (Verheyen & Hermy 2004; Chapter 3). The present study extends this finding with the increased and equal mean number of seeds in *G. urbanum* and *P. elatior*, respectively. The different responses of *P. elatior* and *G. urbanum* to the former land use in terms of the generative performance (including the number of seeds produced) may thus at least partly contribute to their contrasting trends in population growth in post-agricultural forest, i.e., the former species decreasing and the latter species successfully expanding.

4.2 Pot experiment: 3 P levels

Increased P availability in the forest topsoil layer is considered one of the most persistent abiotic legacies of former agricultural land use (e.g., Koerner et al. 1997; Verheyen et al. 1999; Dupouey et al. 2002; Richter et al. 2006; Chapter 1). While a number of studies have demonstrated that the P surplus leads to increased aboveground P uptake and may alter the performance of forest plants (Dupouey et al. 2002; Hipps et al. 2005; Chapters 3–5), its effect on the production of germinable seeds has not yet been studied. The results of the present pot experiment showed that the germination percentage of *P. elatior* seeds significantly increased with increasing P supply (Table 6.2). In Chapter 5, we found a positive effect of P on the size and aboveground biomass of *P. elatior*, but there was no effect on three morphological measures of generative performance (number and height of inflorescences, number of flowers). The present Chapter, however, shows that P does have an effect on the generative performance (germinability) and that the tendency of *P. elatior* seeds to exhibit higher geminability in the post-agricultural experimental populations may therefore be related partly to the higher P availability at those sites. The stronger effect of P on germinability for individuals growing in the pots compared to those growing under field conditions, however, indicates that there are environmental limitations other than P that additionally constrain seed quality in the experimental populations. Yet, it is a fascinating observation that the P surplus is beneficial to seed germinability, but, at the same time, detrimental for seedling and juvenile survival via the increased interspecific competition with the resident vegetation. These patterns are a particular example of so-called life stage conflicts or ontogenetic niche shifts (*sensu* Schupp 1995; Young et al. 2005): the suitability of the local environmental conditions shifts when plants move from one life stage into the next. However, additional research is needed to determine whether the positive effect of P availability on germinability results directly from an effect of P on the seed's ecophysiology or indirectly via the increased performance of the mother plant which may allocate more resources to the reproductive output. Since in wild plants (as opposed to cultivated plants) the seed mineral composition remains generally relatively stable with varying nutrient supply to mother plants, the seed germinability may be mainly related to mother plant performance (Roach & Wulff, 1987; Allison, 2002).

The germination percentage and mean number of seeds per fruit in *G. urbanum* were not affected by P availability. Other environmental differences between ancient and post-agricultural forest are therefore driving the higher seed numbers per fruit and tendency for higher germinability in the latter sites. The significant effect of light availability on the number and height of inflorescences and the number of flowers (Chapter 5) suggests that light may be an important driver. Meekins & McCarthy (2000), for instance, showed that higher light availability resulted in higher numbers of seeds per fruit in another rosette hemicryptophyte forest herb (*Alliaria petiolata* Cavara & Grande).

In sum, this paper has provided experimental evidence that the expansion of founding populations of understorey plant species in post-agricultural forests, at least for the two herbs included here, is not constrained by the production of germinable seeds. Moreover, the species may even profit from the former land use seeing that they tended to increase seed germinability. The differential population trends of the two species in post-agricultural forest stands are therefore attributable to limitations at the post-germination stages of plant recruitment (cf. Chapters 2 and 3). The pot experiment in which we applied three P levels provided further insights into the potential effects of land-use legacies on seed quality and quantity in the recovering forest plant populations. While the increased P availability strongly contributes to the dominance of highly competitive understorey species impeding seedling and juvenile survival in founding populations of forest plants (e.g., Endels et al. 2004; Jacquemyn & Brys 2008), its effect may be rather beneficial for seed germinability. This is just one example of how the abiotic land-use legacies may have differential effects on the successive life stages of colonizing forest plants suggesting that the ultimate effect at the population level might turn out to be very complex.

CHAPTER 7 – UNEXPECTED UNDERSTOREY COMMUNITY DEVELOPMENT AFTER 30 YEARS IN ANCIENT AND POST-AGRICULTURAL FORESTS

AFTER: BAETEN L, HERMY M, VAN DAELE S, VERHEYEN K (2010) UNEXPECTED UNDERSTOREY COMMUNITY DEVELOPMENT AFTER 30 YEARS IN ANCIENT AND POST-AGRICULTURAL FORESTS . *JOURNAL OF ECOLOGY* 98, 1447-1453.

Abstract

Land-use change is considered one of the most radical and extensive disturbances that have influenced plant distributions and diversity patterns in forest understorey communities. In forests growing on former agricultural land, local species diversity and community differentiation among sites are generally reduced compared to ancient forests. Yet, no study has determined how the compositional differences created by former land use change over time as the forest sites recover from former agricultural use. Here we resurveyed 78 vegetation plots (half of the plots in ancient and half in post-agricultural forest) to demonstrate how three decades of forest development have changed the compositional differences between post-agricultural and ancient forest sites. The impact of land-use history and survey date was tested on two measures of species diversity and two measure of community divergence. The data indicate that the imprint of former agricultural land use persisted over time, yet not through compositional stability. Parallel and strong vegetation shifts occurred in both ancient and post-agricultural forest: the species diversity decreased and local species cover strongly diverged, which indicates community drift. The observed understorey changes did thus not support the commonly accepted model of community development in post-agricultural forests, i.e., the diversity did not increase and the vegetation did not become more similar to the ancient forest vegetation over time. The changes in species composition were associated with an increase of common, competitive species at the expense of ancient forest indicator species. The source populations of ancient forest species have been gradually depleted, so the recovery of post-agricultural forests becomes even more precarious. While land-use history is likely to persist as the primary predictor of local species diversity and community divergence, other environmental drivers may additionally structure forest understorey communities and lead to biotic impoverishment and pervasive species reordering on the time scale of only decades.

1 Introduction

The extinction and colonization of species in local plant communities are major community-structuring processes that are profoundly influenced by disturbances (*sensu* Picket & White 1985; Grime 2001) and environmental changes (e.g., Smith et al. 2009). Yet, plant communities often show a delayed response to disturbance or environmental change because of lags in the extinction and colonization of species (Jackson & Sax 2009). Habitat degradation or destruction may directly remove local populations, but species in remnant, isolated habitat patches may experience time-delayed extinction because of a continuing negative population growth. The total number of species that ultimately becomes extinct constitutes the 'extinction debt' (Tilman et al. 1994; Vellend et al. 2006; Jackson & Sax 2009). Likewise, species colonization might be delayed after disturbance because of lag phases in the dispersal and establishment of populations, representing the colonization or immigration credit (Hanski 2000; Jackson & Sax 2009). Furthermore, chronic resource alterations (e.g., increased levels of reactive nitrogen deposition or climate change) represent pervasive environmental changes that might additionally generate gradual extinction and colonization within plant communities. Smith et al. (2009) predict that chronic resource alterations cause a hierarchical community response in which individual species level responses are followed by community reordering, species loss and colonization of species that are better suited for the new environmental conditions.

Massive forest clearance for agriculture followed by land abandonment and forest recovery forms one of the most radical and extensive disturbances in many landscapes in Europe and eastern North-America over the past centuries (e.g., Whitney 1994; Kirby & Watkins 1998). Because the former land use has largely removed the forest vegetation and because many forest understorey plant species have a very limited colonization capacity, local (alpha) diversity in post-agricultural forest may be reduced compared to historically continuous 'ancient' forest for decades to centuries (reviewed in Flinn & Vellend 2005; Hermy & Verheyen 2007; Chapter 1). Beta diversity will also be suppressed in post-agricultural forests because the colonization capacity of forest plants is strongly limited by dispersal (e.g., Matlack 1994; Verheyen et al. 2003c) and the same suite of good dispersers of the regional pool will, consequently, be overrepresented in the local community relative to the weak dispersers (Vellend 2004; Vellend et al. 2007). Furthermore, the remnant vegetation in isolated ancient forest patches may lose species over time resulting from stochastic extinction or negative demographic rates, i.e., paying off their extinction debt (Vellend et al. 2006; Rogers et al. 2009). In this way, land-use history may have an overriding impact on the vegetation patterns in present day forests (Flinn & Vellend 2005).

There is strong evidence that forest vegetation is also prone to dramatic shifts on a time scale of decades driven by the chronic alteration of the environmental conditions (e.g., Thimonier et al. 1994; Rooney et al. 2004; Taverna et al. 2005; Wiegmann & Waller 2006; Rogers et al. 2008; Van Calster et al. 2007; Baeten et al. 2009). The observed vegetation changes in the cited studies generally fit the hierarchical response framework suggested by Smith et al. (2009), i.e., communities are strongly reordered in terms of relative abundances and habitat specialists are largely replaced by already widespread species. The shifts are thus generally associated with biotic impoverishment and increased similarity among habitat patches, called biotic homogenization *sensu* Olden & Rooney (2006). Although forest vegetation responses to land-use history on the one hand and gradual responses to chronic environmental changes on the other hand are likely to occur simultaneously, they have never been studied and discussed collectively. The divergent ways of quantifying the two responses, i.e., land-use effects in contemporary landscapes versus temporal comparison using (semi-)permanent plots, largely account for this research gap. A combination of both approaches might, however, be particularly insightful into the patterns of successional recovery in post-agricultural sites within a backdrop of gradual forest herb community shifts.

One of the pioneer studies describing the overriding impact of land-use history on forest plant distributions was performed in forests in the western part of Belgium and northern France about thirty years ago (Hermy & Stieperaere 1981; Hermy 1985). We used topographical maps and detailed field descriptions to relocate 78 of the former vegetation plots in ancient (39 plots) and post-agricultural forest (39 plots) and we performed a resurvey of the vegetation. In this way, we were able to determine the actual long term colonization of forest plants into post-agricultural forests, which integrates the different stages analysed in Chapters 2–6. The data were analyzed using two measures of species diversity and two measures of community divergence. We hypothesized that partial vegetation recovery from past agriculture reduced the compositional differences between post-agricultural versus ancient forest sites over time, but, at the same time, gradual changes in vegetation composition led to overall impoverishment and homogenization within and between sites, respectively.

2 Methods

2.1 Study area, plot selection & forest community surveys

The studied forests are all situated in the former County of Flanders, comprising the northwestern part of Belgium, western Europe (Fig. 7.1). It is a level area with altitudes varying between 0 and 40 m a.s.l.; soil texture varies from sandy in the north to loam in the south. The history and distribution of forests in the landscape is the result of several phases of forest progression and regression (Tack et al. 1993; Verheyen et al. 1999). The earliest traces of human impacts on the forests in the County go back to prehistorical times, and there is clear evidence for deforestation for agriculture and reforestation during Roman times (50 BC–350 AC) and the Middle Ages (4th century–14th century). The first regional, detailed (scale *c.* 1/12500) land-use map was constructed around 1775 (De Ferraris map). Forest cover equalled about 12.8 % at that time, but decreased to 4.7 % by 1880 (Tack et al. 1993). Afterwards, the forest area slightly increased again and forest cover in the present day landscape is about 5 %.

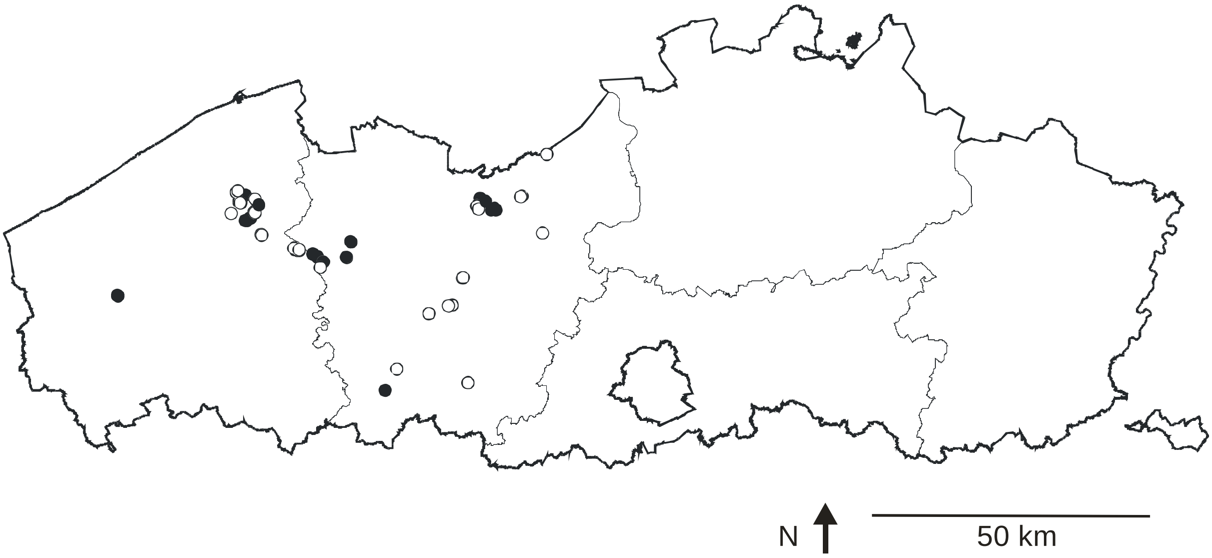


Fig. 7.1 Study region in the western part of northern Belgium. Plot locations are indicated by open (ancient forest) and filled symbols (post-agricultural forest).

Between 1977 and 1983, M. Hermy recorded the forest vegetation in 640 vegetation plots (100–200 m²) distributed over 183 forests in the study region (Hermy 1985). The data from this survey will be further referred to as the 1980 data. The percentage cover of each species in the herb layer was estimated using Londo's decimal scale during late spring (April–first half of June). The plots were marked on topographic maps (scale 1/25 000) and additional descriptions and location sketches were recorded on individual fiches. Land-use history of the forest stands was reconstructed using the map of De Ferraris (1775) and the first edition (1862) and revised version (1884–1911) of the Ordnance Survey maps of Belgium. The aim of this dataset was to work out a phytosociological classification of ancient and post-agricultural forest vegetation in the region (Hermy 1985). For the present study, we selected 78 plots based on phytosociological and historical-ecological criteria. First, the plots had to be situated in alluvial forest, a forest type that supports species-rich communities and is of particular concern for conservation in Europe (protected with priority in the European Habitat Directive). Second, selected plots lay in ancient forest, i.e., continuously forested since the oldest land-use map of De Ferraris (1775), or in forest established on former agricultural land after 1862 (first topographical map). This pragmatic classification is based on the common definition of ancient and post-agricultural forest (see Flinn & Vellend 2005), and fits the last afforestation movement in the region (see above). Third, the plots were in forest stands with no recent cuttings (< 10 years); this was evaluated in the field.

In late spring 2009, we relocated the 78 selected plots using the topographical maps, field descriptions and guidance of the original surveyor (M. Hermy). Half of the plots were in ancient forest stands, half in post-agricultural stands. While the plots were thus not permanently marked in the field, the procedure has proven to result in reliable temporal comparisons in previous projects (e.g., Van Calster et al. 2007; Baeten et al. 2009). After relocating a plot, we recorded the vegetation of the herbaceous layer analogously to the old survey, i.e., we used the same plot sizes and cover scale. Individuals of the genera *Viola* and *Rubus* were not determined at the species level.

2.2 Data analysis

We applied four community measures that were proposed by Vellend (2004) in a study that determined land-use effects on the species and genetic diversity of forest plants. Two measures of species diversity and two measures of community diversity were calculated using presence-absence data and cover data. The two measure for species diversity were the number of species in a plot, i.e., Species Richness (SR, presence-absence data), and Evenness

(E, cover data). For the calculation of E, we first rescaled (normalized) the percentage cover of each species so that the sum of the rescaled cover values of all species in a plot equalled one (NC_i: normalized cover of species i; $\Sigma NC_i = 1$). Then, E was calculated as $1 - \Sigma(NC_i)^2$. The measure E can be interpreted as the probability that two randomly chosen single-species patches that represent one percent of the total cover will belong to a different species. We used the odds ratio E/(1-E) in the statistical analyses to remove the positive skew in the distribution of E.

The first measure of community divergence was the presence-absence based Raup-Crick beta diversity (β_{RC}). This is a probabilistic measure of beta diversity that is not biased by differences in species richness among plots, i.e., it is suitable to determine changes in divergence isolated from changes in diversity. See Vellend (2004) and Vellend et al. (2007) for a good argumentation on this topic. The β_{RC} index gives the probability that two plots share fewer species than expected under a null model. First, the number of species shared by plots *x* and *y* with *i* and *j* species, respectively, is calculated. Then, we take 1000 random draws of *i* and *j* species from the 'species pool', which is the collection of all species in the 78 plots. The probability of selecting a species is proportional to the number of plots in which it occurred. The proportion of pairs of null communities that have the same number or more species in common than plot *x* and *y* represents the divergence between those plot ($\beta_{RC,xy}$). For a given plot, the mean of all the pairwise values against all other plots is that plot's degree of community divergence or β_{RC}. The calcultation of β_{RC} for each plot was done for the 1980 data and the 2009 data separately, thus, it gives the degree of community divergence of a plot in 1980 or 2009 against all other plots in the landscape at that time.

The second measure of community divergence (F_{ST}, cover data) was a community analogue of the fixation index in population genetics (F_{ST}), which expresses the reduction in heterozygosity in a subpopulation due to genetic drift. In community terms, the index describes differences in the relative cover of species between plots, i.e., a weak correlation between the species cover in two plots indicates divergence in local abundance or 'community drift'. The divergence between two plots *x* and *y* is based on the Evenness index (see above) and is calculated as $F_{ST,xy} = (E_{between} - E_{within})/E_{between}$. For the pair of plots, $E_{between}$ is the Evenness index based on summed cover values of each species in the two plots. The

E_{within} is simply the mean of the Evenness values for each of the two plots. The F_{ST} value for a given plot is the mean of all the pairwise values against all other plots.

We used Generalized Linear Mixed Models (GLMMs) to test the effect of former land use (ancient vs. post-agricultural) and survey year (1980 vs. 2009) on the four diversity indices. Plot identity was used as a random factor to account for the paired nature of the plots over time. Models for the response variables E/(1-E), β_{RC} and F_{ST} were fitted using the *lme* function of the *nlme* package in R (Pinheiro et al. 2009; R Development Core Team 2009). Because species richness (SR) is a count variable, we applied Poisson GLMM with log link function implemented in the *lmer* function in the *lme4* package (Bates & Maechler 2010).

Analysis of Dissimilarity (ADONIS) was used to test whether the differences in community composition between the two time periods and between plots with contrasting land-use history were significant (Anderson 2001). The interaction term between land use and survey indicates whether the community composition in post-agricultural and ancient forest became more similar over time. The analysis was performed on the full untransformed data matrix and the Jaccard distance on species cover data was used to calculate dissimilarities among plots. Calculations were done in the *vegan* package in R. Finally, we determined which species or species strategies mainly accounted for the community changes between 1980 and 2009. First, we focused on the species that were significantly associated with ancient forest in 1980 (ancient forest species *sensu* Hermy et al. 1999) because those species largely account for the persistent differences in community composition between ancient and post-agricultural forest. Species associations were tested using the chi^2-square statistic on species frequencies (cf. Hermy & Stieperaere 1981; Hermy 1985). The change in frequency of these species between 1980 and 2008 was tested with a Wilcoxon Signed-Rank test with Monte Carlo determination of the significance. Second, we calculated the C-S-R signature of each plot in 1980 and 2009 according to Hunt et al. (2004). The signature is a three-part numerical index (C, S and R coordinate) that represents the balance between the plant strategies (*sensu* Grime 2001) within the community based on the cover of individual species. Changes between 1980 and 2009 in ancient or post-agricultural forest were tested with paired t-tests.

3 Results

We found 105 different species across the 78 plots in the old survey and 94 species in the new survey. Twenty-nine species were only found in the old survey and 18 new species appeared in the new survey. The ADONIS showed that land-use history was a stronger predictor for the variation in community composition among sites (F = 5.938, $P < 0.001$) than survey date (F = 3.081, $P < 0.001$). The interaction between land use and survey was not significant (F = 1.268, $P = 0.172$), i.e., the post-agricultural forest vegetation did not become more similar to the ancient forest vegetation over time.

Land-use history had a significant impact on the species diversity and community divergence (Table 7.1; Fig. 7.2). The evenness (E) and presence absence based community divergence (β_{RC}) were reduced in post-agricultural versus ancient forest stands, while cover based divergence (F_{ST}) was significantly higher in post-agricultural forest. Furthermore, the two measures of species diversity (evenness and species richness) decreased between 1980 and 2009. The cover based community (F_{ST}) divergence increased over time, i.e., local species cover appears to have diverged. The species that showed the strongest shifts in plot-level cover were all generalists characterized by a competitive component in their strategy (see Appendix 7.1). The interaction between land use and survey was never significant.

Table 7.1 Effect of Survey (1980 vs. 2009) and Land-use history (ancient vs. post-agricultural) on two measures of species diversity and two measures of community divergence in 78 vegetation plots in alluvial forest sites in the western part of Belgium.

	Survey			Land use			Survey × Land use	
	Effect	F/z	*P*	Effect	F/z	*P*	F/z	*P*
Diversity								
SR	–	-3.122	0.002		0.623	0.533	-0.630	0.529
E/(1-E)	–	5.944	0.017	–	2.911	0.092	1.753	0.190
Divergence								
β_{RC}		0.007	0.934	–	5.372	0.023	2.689	0.105
F_{ST}	+	22.408	< 0.001	+	4.560	0.036	1.138	0.290

Notes: Generlized Linear Mixed Models included plot identity as random factor to account for the paired nature of the data. Survey (1980 = 0; 2009 = 1) and Land use (ancient = 0; post-agricultural = 1) were coded as binary variables, thus positive effects indicate higher values in 2009 or post-agricultural sites. Effects are shown if $P < 0.1$. The response variables E/(1-E) and β_{RC} were square root and log transformed, respectively.

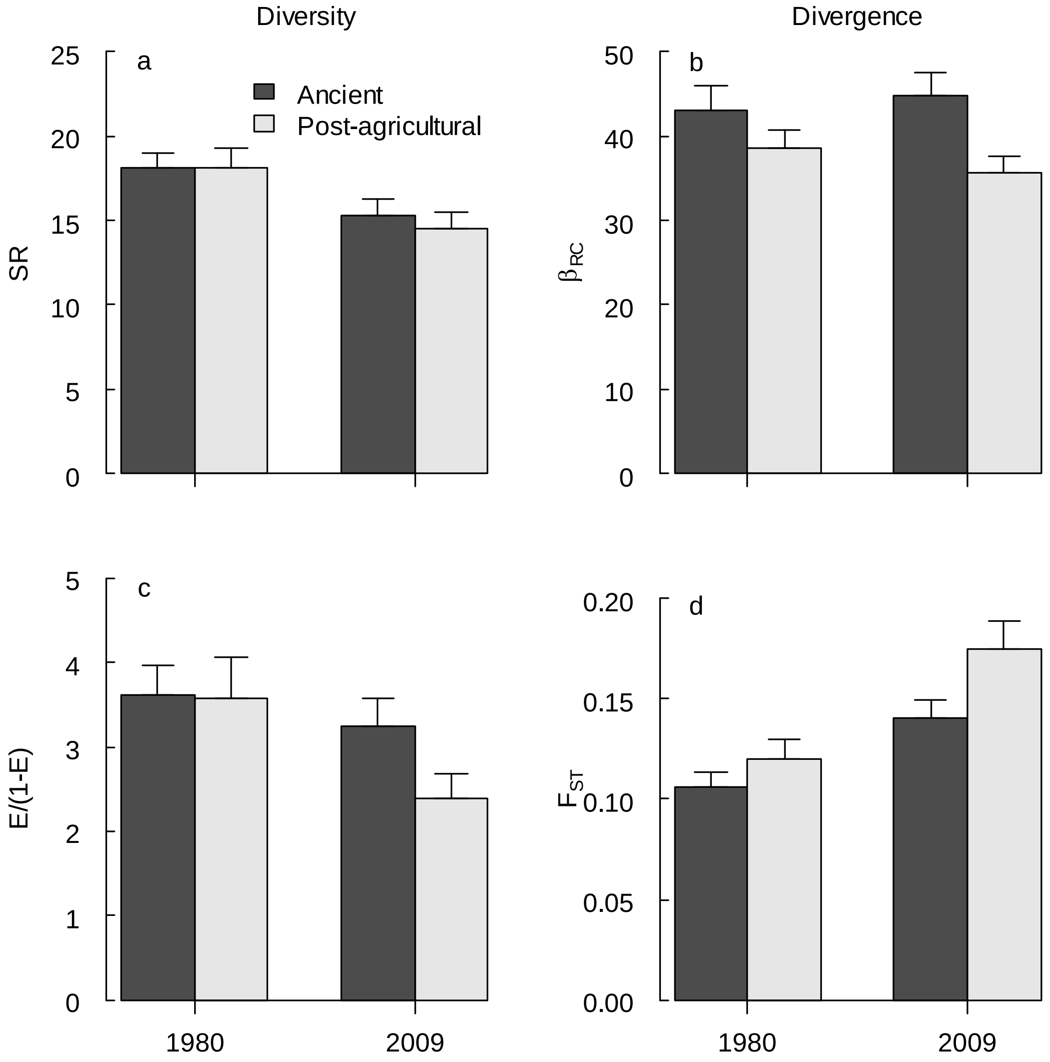


Fig. 7.2 Effect of land-use history and survey on the two measures of species diversity (a, c) and the two measures of community divergence (b, d). SR: Species Richness; E/(1-E): odds ratio of Evenness index (E); β_{RC}: presence-absence based community divergence (Raup-Crick beta diversity); F_{ST}: cover based community divergence. Statistical results are reported in Table 7.1.

We identified nine forest plants that were significantly associated with ancient forests (chi²-test $P < 0.1$; inset Fig. 7.3). Seven out of the nine ancient forest species contributed to the decline in species richness of the ancient forest sites (Fig. 7.3; change in frequency Z = -1.66, $P = 0.05$). These species disappeared on average from 31 % of the plots in which they occurred in 1980. The analysis of the C-S-R signature of plots showed that the C-component significantly increased (t = 2.94, $P = 0.006$), while the R-component (t = -1.99, $P = 0.05$) and S-component (t = -1.91, $P = 0.06$) decreased in ancient forest (Appendix 7.2). In post-agricultural forest, only the R component significantly decreased (t = -2.17, $P = 0.04$).

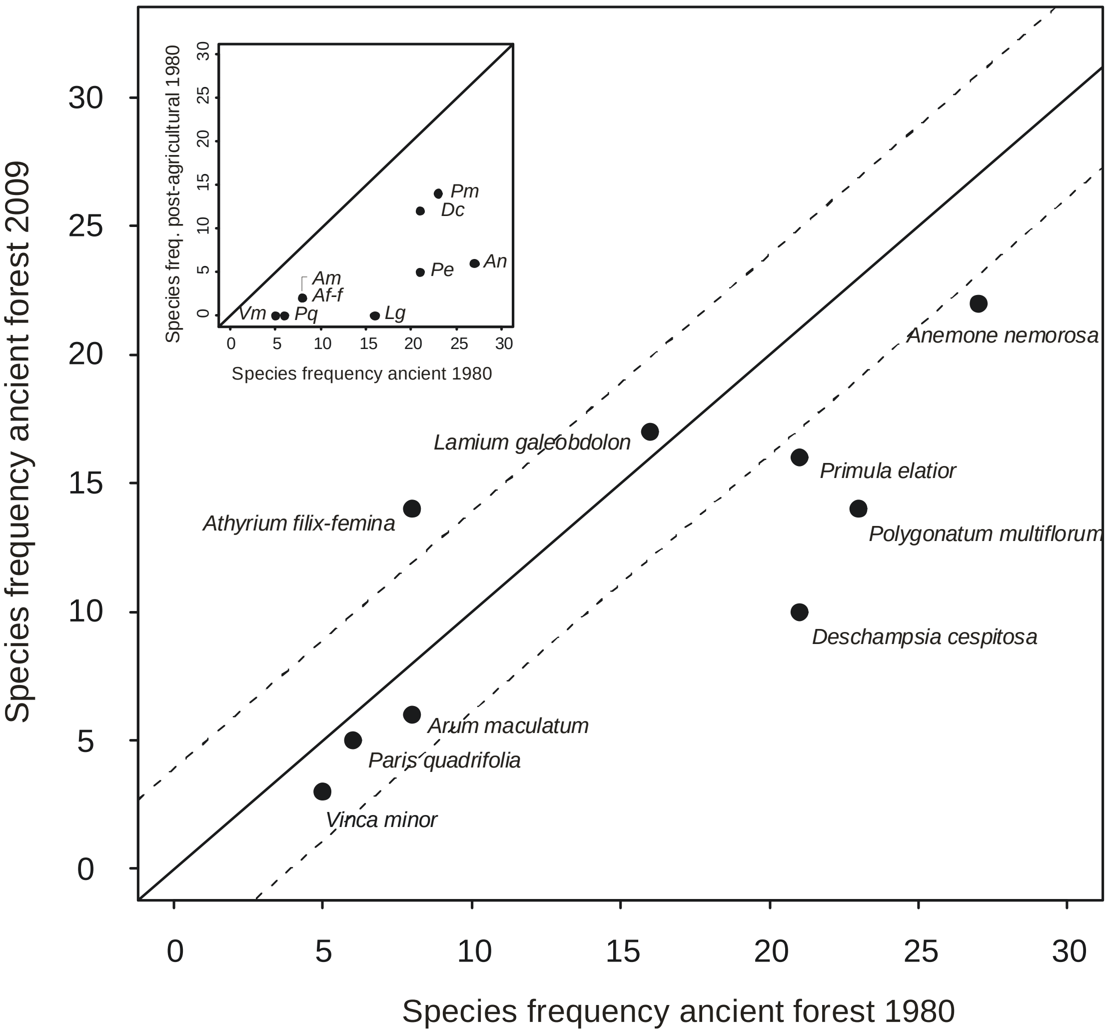


Fig. 7.3 Change in frequency of species that were significantly associated with ancient forest ('ancient forest species') between 1980 and 2009 (n = 39). The full line represents the 1:1 line of no change, the dashed line a change in frequency of 10 % of the 39 plots. The inset figure gives the frequency of the species (abbreviated) in ancient forest vs. post-agricultural forest in 1980.

4 Discussion

The patterns of diversity and divergence of the forest herb layer communities were clearly related to former land use, but also changed significantly over time. Moreover, the effects of land use and survey date were additive, i.e., effect directions were concordant and interactions were never significant. The temporal shifts in post-agricultural forest vegetation thus largely parallel the shifts in ancient forest vegetation, which means that the community differences created by former land use persisted over time. Furthermore, land use was a stronger predictor of community composition than survey date, but both had a significant and additive impact on the variation in species composition among plots. Although we found gradual vegetation shifts over three decades, they do not corroborate the expected vegetation recovery after land-use change and the decrease in compositional differences between post-agricultural and ancient forest sites.

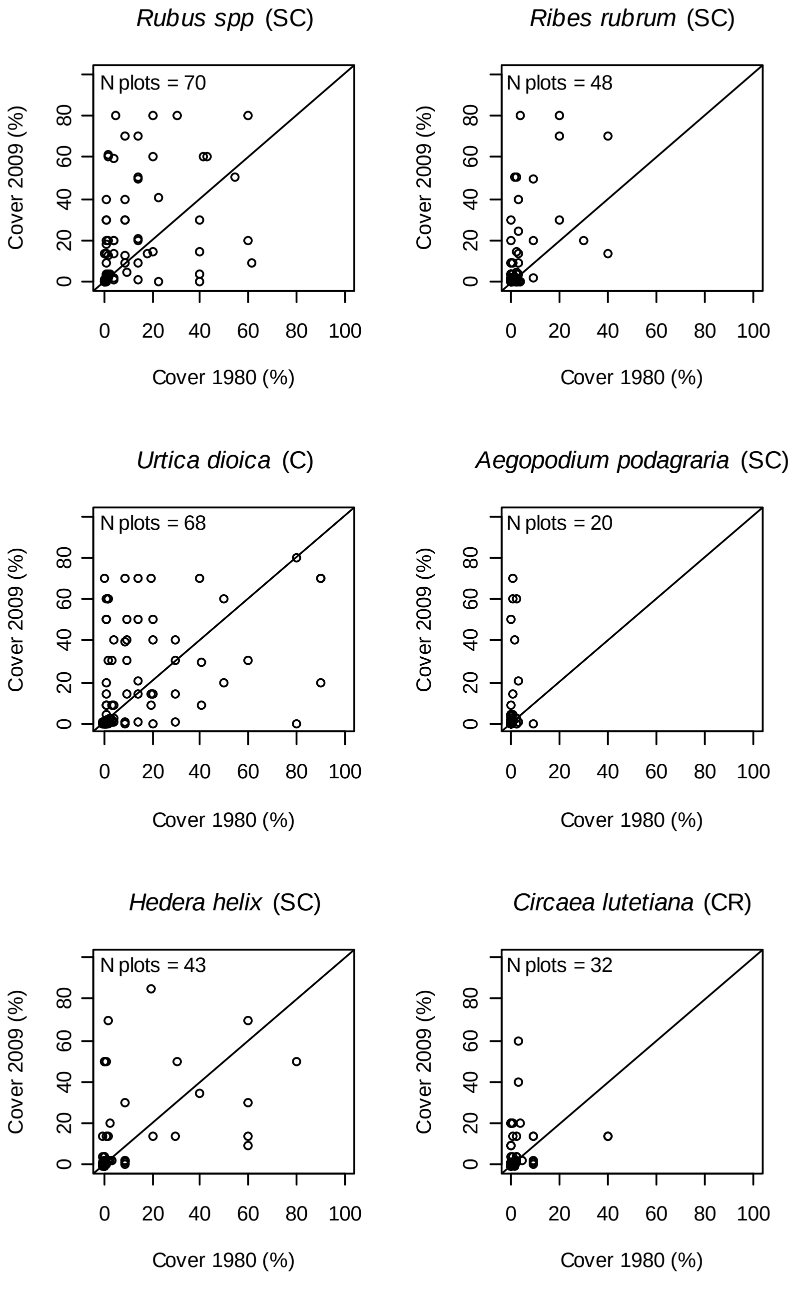
The effects of land use on species diversity and community divergence were largely in accordance with the patterns found by Vellend (2004) in forest stands in central New York (USA). Reduced beta diversity (β_{RC}) in post-agricultural forest compared to ancient forest indicates that the same suite of species tend to be overrepresented in post-agricultural sites. Vellend et al. (2007) suggested that selective dispersal filters constrain the pool of species that can colonize after the vegetation has been cleared away for agriculture, which creates increased similarity among post-agricultural communities, i.e., biotic homogenization (Olden & Rooney 2006). Although a differential dispersal capacity may largely account for the contrasting colonization capacity of forest plants (reviewed in Verheyen et al. 2003c), differences in establishment success may additionally constrain the post-dispersal stages of colonization (Flinn 2007; Chapters 2 and 3). We identified nine forest plants that were significantly associated with ancient forests, of which seven were included in a European list of ancient forest indicators (Hermy et al. 1999). These ancient forest species are pivotal in the studied landscape as they strongly contribute to the differences in composition and beta diversity between ancient and post-agricultural forest vegetation. In contrast to β_{RC}, the divergence in relative species cover (F_{ST}) was the highest in post-agricultural forest. So, while the vegetation composition among post-agricultural sites is relatively similar, local species cover may vary considerably, i.e., species rank differently in terms of their cover among plots. The randomness of local species cover is primarily driven by 'community drift' (*sensu* Vellend 2010) and is expected particularly in biotically impoverished communities (Vellend 2004, 2010). As a suite of dispersal and establishment bottlenecks increases the proportion of ecological equivalent species in post-agricultural forest (cf. Verheyen et al. 2003c; Vellend et al. 2007), demographic stochasticity may have partly replaced interspecific interactions in structuring local abundances (Hubbel 2001).

Species diversity, measured as species richness and evenness, significantly decreased between 1980 and 2009. This observation contradicts the widely accepted model of community development as forests recover from past agriculture, i.e., diversity gradually increasing over time (reviewed in Flinn & Vellend 2005). A decline in (native) species diversity has also been observed elsewhere in European and North American forests and was attributed to canopy succession (Brewer 1980; Rogers et al. 2008; Van Calster et al. 2008a) and to gradual alterations of the environmental conditions including changes in grazing, forest management, fire regime and atmospheric deposition and the increase in non-native species invasion (e.g., Thimonier et al. 1994; Rooney et al. 2004; Taverna et al. 2005; Rogers et al. 2008; Van Calster et al. 2007, 2008a). The cause of species decline in the present study is probably a combination of the stand maturation (e.g., increased shade) that has followed the decline of traditional, intensive management (Tack et al. 1993; cf. Taverna et al. 2005) and the desiccation and eutrophication associated with the intensification of the land use in the surrounding landscape (Lameire et al. 2000). The overall decline of species with ruderal component in their strategy and simultaneous increase of competitive species (Appendix 7.2), for instance, suggest a lower disturbance level (relaxation of management) and increased nutrient availability by eutrophication (Grime 2001). Furthermore, seven out of the nine ancient forest species disappeared on average from one third of the plots between 1980 and 2009 and contributed to the decline in species richness of the ancient forest sites. The demanding nature of these forest specialists probably underlies their sensitivity to local extinction under changing habitat conditions. Species relaxation after historical forest fragmentation, i.e., paying off the extinction dept, may also contribute to the losses (Vellend et al. 2006; Rogers et al. 2009; Jackson & Sax 2009). At the same time, only two out of the nine species colonized more than one post-agricultural plot over thirty years. A colonization credit thus still persists several decades after agricultural abandonment. As ancient forest populations appear to decline quite rapidly, source populations are progressively depleted and the slow recovery of post-agricultural forest vegetation becomes even more precarious.

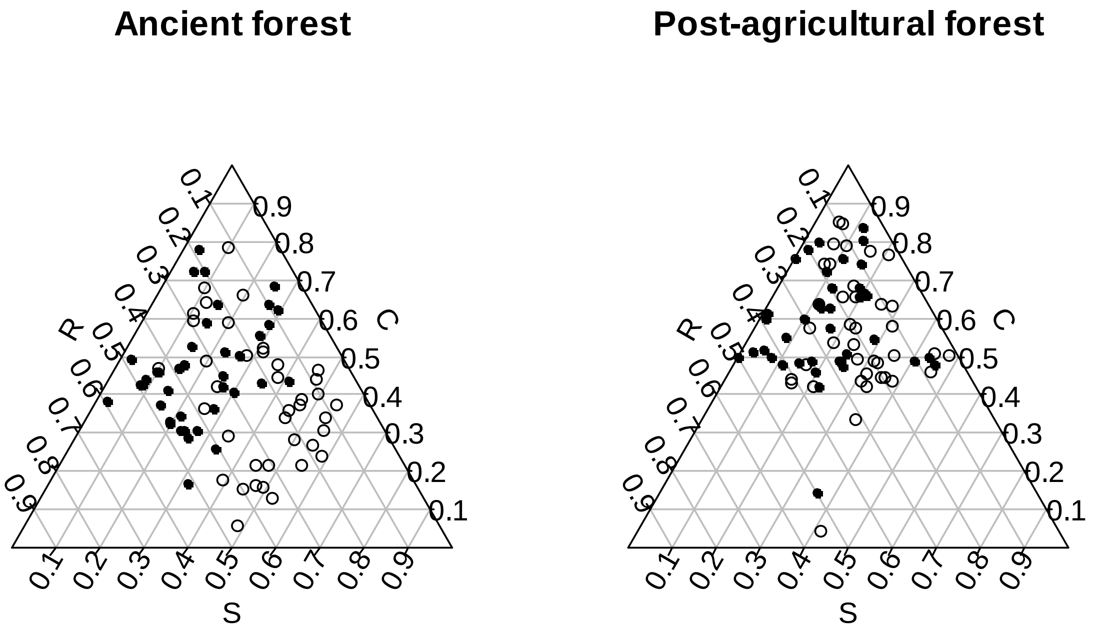
The divergence among plots in terms of species composition (β_{RC}) did not change between 1980 and 2009, so forest herb layer communities did not suffer from the temporal biotic homogenization reported in other studies (e.g., Rooney et al. 2004; Rogers et al. 2008; Van Calster et al. 2007). Yet, the divergence in terms of local species cover (F_{ST}) did increase considerably over time, i.e., different species gained dominance in different plots. Local cover did, however, not diverge completely randomly: the species exhibiting the strongest changes in absolute cover are all characterized by a competitive component in their plant strategy *sensu* Grime (2001) and generally increased their cover (Appendix 7.1). A common feature of those species (e.g., *Rubus fruticosus* spp*, Ribes rubrum* L.*, Urtica dioica* L.*, Aegopodium podagraria* L. and *Hedera helix* L.) is their capacity for rapid growth, high biomass production and fast lateral spread, which enables them to produce high shoot and root densities and to outcompete other forest plants. Thus, although the identity of the species that increased in each plot differed, the nature of those species was relatively similar among plots. The herb layer communities in the present study appear to have entered the species reordering stage predicted by the hierarchical response framework of Smith et al. (2009). At this stage, a particular suite of species, here the species with stronger competitive ability, are being favoured by changing environmental conditions at the expense of others. The species reordering stage is expected to result in a subsequent phase associated with species extinctions and the immigration of generalists (Smith et al. 2009). Although no biotic homogenization has been observed yet, future compositional changes are to be expected.

To conclude, the overriding impact of land-use history on local species diversity and community divergence of the forest herb vegetation persisted for thirty years. Thus, unlike the commonly accepted model of community development in post-agricultural forests, species diversity and compositional similarity with ancient forest did not increase over time. The permanence of land-use legacies was not simply the result of compositional stability; we found strong and parallel declines in local species diversity and increased divergence in species cover in both ancient and post-agricultural forest sites. Jackson & Sax (2009) recently conceived the ‘biodiversity balance’, which is the diversity level after the extinction debt or the colonization credit following a major disturbance have been paid off (e.g., habitat fragmentation or destruction). Our results not only show that the forest communities have not yet achieved this equilibrium following the former land-use changes, but also indicate that the overall diversity level in both ancient and post-agricultural forest has strongly declined in a time span of decades due to biotic impoverishment and profound species reordering. Although knowledge about the former land use enables us to largely explain the distributional patterns and cover of species in contemporary landscapes, the resurvey of historical records provides important additional insights into how present-day plant communities were structured during the past decades and how they might shift in the future.

Appendix 7.1 Plot-level changes in species cover (%) for a selection of six species that showed the largest mean absolute change in plot-level cover between 1980 and 2009. The strategy of the species is reported within parenthesis (Hodgson *et al.* 1995). The "N plots" gives the total number of plots in which the species occurred (total number = 78) in at least one of the two survey years (1980 or 2009).



Appendix 7.2 Changes between the C-S-R signature of vegetation in 1980 (open circles) and 2009 (filled circles). The left figure shows the plots in ancient forest, the right figure in post-agricultural forest. The C-S-R signature of a plot is a three-part numerical index (C, S and R coordinate) that represents the balance between the plant strategies (*sensu* Grime 2001) within the community based on the cover of individual species. The signature was calculated according to Hunt et al. (2004). For ancient forest vegetation, a paired t-test showed that the C-component increased (t = 2.94, *P* = 0.006) at the expense of the R-component (t = -1.99, *P* = 0.05) and S-component (t = -1.91, *P* = 0.06). For post-agricultural vegetation, only de R component significantly decreased (t = -2.17, *P* = 0.04).



Appendix 7.3 Species-level changes in frequency and cover between 1980 and 2009. Only species occurring in 10 % of the plots in the entire dataset (1980 and 2009 data) are reported.

Species	Frequency		Cover	
	1980	2009	1980	2009
Rubus fruticosus agg.	70	69	12	23
Urtica dioica	68	63	17	27
Poa trivialis	53	31	2	3
Glechoma hederacea	51	54	13	12
Geum urbanum	51	45	1	3
Filipendula ulmaria	49	24	4	2
Moehringia trinervia	48	31	2	2
Galium aparine	47	56	2	3
Galeopsis tetrahit	46	36	1	1
Cardamine pratensis	43	12	2	3
Cirsium palustris	40	11	1	1
Polygonatum multiflorum	37	21	2	1
Rumex sanguineus	37	18	1	1
Ribes rubrum	33	48	8	16
Anemone nemorosa	33	34	27	15
Deschampsia cespitosa	33	18	6	5
Adoxa moschatellina	32	32	4	5
Angelica sylvestris	27	22	1	2
Primula elatior	27	21	4	3
Hedera helix	26	43	20	17
Stachys sylvatica	26	28	2	1
Circaea lutetiana	25	32	7	10
Viola spp	23	10	1	2
Lonicera periclymenum	22	25	1	2
Valeriana officinalis	21	7	1	2
Ajuga reptans	19	13	1	2
Holcus lanatus	19	9	4	4
Scrophularia nodosa	19	8	1	1
Carex sylvatica	18	18	2	1
Carex remota	17	18	1	8
Lamium galeobdolon	16	18	36	23
Humulus lupulus	16	15	4	1
Symphytum officinale	15	13	1	2
Aegopodium podagraria	13	20	2	18
Listera ovata	13	3	2	1
Lysimachia vulgaris	13	2	2	2
Iris pseudoacorus	12	9	1	1
Lysimachia palustre	12	6	1	1
Galium palustre	12	5	1	1
Athyrium filix-femina	10	23	2	1
Arum maculatum	10	7	2	1
Solanum dulcamara	10	2	2	2

Geranium robertianum	9	13	1	2
Ranunculus repens	9	6	3	1
Ornithogalum umbellatum	9	3	1	1
Brachypodium sylvaticum	9	2	2	2
Poa nemoralis	8	1	1	1
Heracleaum sphondylium	7	7	1	1
Paris quadrifolia	6	6	2	4
Lasana communis	5	7	1	1
Scutellaria galericulata	5	4	3	1
Taraxacum officinalis agg.	5	4	1	1
Carex elongata	5	3	1	1
Vinca minor	5	3	13	33
Dryopteris dilatata	4	27	1	4
Dryopteris carthusiana	4	8	3	1
Epipactis helleborine	3	9	1	1
Juncus effusus	3	8	1	1
Dryopteris filix-mas	3	7	1	1

CHAPTER 8 – GENERAL DISCUSSION AND CONCLUSIONS

Both dispersal and establishment may limit the distribution and abundance of forest understorey plant species (Eriksson & Ehrlén 1992; Clark et al. 2007; Chapter 1). Within the context of forest plants that colonize post-agricultural forests, most research has focused on dispersal limitation and, in some cases, the relative importance of dispersal versus establishment limitation (e.g., Donohue et al. 2000; Graae 2004; Verheyen & Hermy 2001b, 2004). The causes and consequences of establishment limitation received much less attention. Here we divided plant establishment into different components (stages of recruitment, adult performance and reproduction) and determined which particular component may impede the colonization of forest plants into post-agricultural forests (see Fig. 1.3). While different legacies of the past agriculture may cause the establishment success to differ between post-agricultural and ancient forest sites, we mainly concentrated on the impact of former fertilization (P availability in particular). The present chapter summarizes our main findings on plant recruitment (Chapters 2 and 3), subsequent adult plant performance (Chapters 4 and 5) and reproduction (Chapter 6). Based on this summary and the results of Chapter 7, we propose a synthetic framework for community development in post-agricultural forests. We end the discussion by presenting some management consequences and directions for further research.

1 Land-use effects on forest plant establishment

1.1 Recruitment into the adult life stage

We sowed seeds of ten forest understorey plant species at three densities in post-agricultural forest sites (alluvial and plateau) and monitored their germination and recruitment into the adult life stage for five growing seasons (Chapter 2). After two growing seasons, recruits of each introduced species occurred in the community and, what is more, adding seeds at higher densities generally resulted in more recruits. These findings, together with the results of other introduction experiments on forest understorey plant species (e.g., Ehrlén & Eriksson 2000; Graae et al. 2004; Verheyen & Hermy 2004; Ehrlén et al. 2006; Chapter 3), demonstrate that a restricted propagule pressure (i.e., dispersal limitation) controls plant colonization. Yet, a second key result of our experiment was that only a small fraction of the seeds effectively recruited: for most species, less than 5 % of the seeds recruited into the seedling stage after two growing seasons (cf. Verheyen & Hermy 2004). Furthermore, the subsequent mortality further decreased the number of seedlings that reached the adult life stage after five growing seasons. The recruitment phase thus also controls the colonization of plants, which corroborates the results of the recent review paper by Clark et al. (2007). Because the actual recruitment success is very low, a high seed pressure is needed for plant colonization. However, the low dispersability of many forest plant species coupled with their scattered distribution in fragmented landscapes hampers the seed availability.

We not only found that the overall recruitment of forest plants was low, but also that land-use history may have a significant, but species-specific effect on the recruitment success. The number of adults of *Primula elatior* Hill was significantly higher in ancient compared to post-agricultural forest sites eight years after sowing (Chapter 3). The results for *Geum urbanum* L. were exactly opposite. This finding nuanced the initial patterns reported by Verheyen & Hermy (2004), who found no effect of land use on seedling occurrence after two growing seasons. An important outcome of the experiment was that the slow colonization of *P. elatior* into post-agricultural forest sites (e.g., Hermy et al. 1999; Verheyen et al. 2003c) appears to be partly related to its low recruitment success following seedling establishment. In a modelling study, Jacquemyn & Brys (2008) came to the same conclusion. The high colonization capacity of *G. urbanum* was in accordance with its relatively good recruitment in post-agricultural forest sites. Similarly, Flinn (2007) showed that the establishment rates of three fern species differed as the species colonization capacities would predict, with the fast colonizer having the highest rates and the slow colonizer the lowest.

Interspecific competition with the established vegetation contributed to the overall low recruitment and partly accounted for the impact of former agricultural land use on species colonization. Observational studies already suggested the detrimental impact of competition on plant colonization (e.g., Hermy et al. 1993; Verheyen & Hermy 2001a; Endels et al. 2004; Jacquemyn & Brys 2008), but we provided experimental evidence. Clearing the understorey vegetation increased the recruitment of most aestival and evergreen species. Vernal species such as *Anemone nemorosa* L. and *Hyacinthoides non-scripta* (L.) Chouard complete most of their growth ahead of their competitors, and they were unaffected by the clearing treatments (cf. Verheyen & Hermy 2004). In forest communities with a sparse understorey layer (dry plateau forest), clearing had little or no effect, i.e., interspecific competition might be less important at these sites (Fraterrigo et al. 2006; Chapter 2). Alluvial forests are characterized by rich soil conditions and the P legacy of past agriculture further increases productivity, which allows competitive species to grow vigorously. Evidence from Chapter 4 and other studies (e.g., Pigott 1971; De Keersmaeker et al. 2004; Hipps et al. 2005), for instance, shows the strong effect of P availability on the biomass production of the highly competitive species *Urtica dioica* L. Recruits of the introduced species probably suffered defeat in the asymmetric competition for light with the vigorous vegetation. Species of high-nutrient environments such as *U. dioica* and *Aegopodium podagraria* L. share a suite of traits (e.g., high relative growth rate, horizontal leaf position, relatively large plant height) that reflect adaptation to light competition, which is a race to the top in order to project one’s leaves above those of neighbouring individuals (Craine 2005, 2009). While Hautier et al. (2009) showed that competition for light controls the diversity in eutrophied grassland communities, evidence for forest is still largely lacking.

1.2 Adult performance

The growth, nutrient content and longevity of forest plants may vary with land-use history because the environmental legacies of the former agriculture can have a direct (via phenotypic plasticity) and indirect impact (via altered interspecific and multitrophic interactions) on the performance of forest plants. Reduced performance in post-agricultural compared to ancient forest would suggest that the altered environmental conditions contribute to the slow colonization of forest plants (e.g., Vellend 2005), but we generally found no negative direct effects of former agricultural land use (but see the results for *A. nemorosa* in Chapters 4 and 5). Many forest herbs displayed a positive response to land-use change with a higher performance in post-agricultural relative to ancient forest sites (Chapters 3 and 4; Endels et al. 2004; Verheyen & Hermy 2004; Fraterrigo et al. 2006). Results from pot experiments showed that the increased P availability partly accounts for this land-use effect for some, but not for all forest species (Chapter 5; Pigott & Taylor 1964; Hipps et al. 2005). We found that *P. elatior* and *Circaea lutetiana* L. clearly benefited from higher P availability whereas *G. urbanum* and *A. nemorosa* were relatively unaffected.

We focussed on the P legacy of former agriculture, but other soil nutrients can also contribute to land-use effects on plant performance (see Chapter 1 §2.1). The availability of N as an important nutrient may be of particular interest. In the present thesis we found, however, that while all species realized a higher P uptake in post-agricultural sites, the N concentrations in the aboveground biomass did not depend on land-use history (Chapter 4). We therefore hypothesized that the nutrient might not be persistently altered by the former agriculture or that N is available in excess in the studied alluvial forest systems. Excess N availability is likely to be a peculiarity of alluvial forests with fast litter decomposition and alluvial inputs through inundation. High atmospheric N deposition further increases N availability. In sites where N is limiting plant growth, e.g., in other forest types or in regions with much lower N deposition, land-use effects on the nutrient may alter plant performance (e.g., Fraterrigo et al. 2009).

We also observed unfavourable effects of past agriculture on the performance of forest plants and those effects were at least partly related to the strong interspecific competition with the abundant vegetation in post-agricultural sites. Introduced adults of *P. elatior*, for instance, initially performed better in post-agricultural compared to ancient forest, but the individuals could not sustain this higher performance after several years of growth in the established competitive vegetation (Chapter 3). Strong competition might also account for the reduced longevity of *P. elatior* and *G. urbanum* in our post-agricultural forest sites. But how can these observations be reconciled with the beneficial direct effects discussed above? We suggest that slowly colonizing forest herbs (e.g., *P. elatior*) may well have an inherent capacity to profit from the altered soil conditions, but, at the same time, their growth and longevity is impeded by species that show a stronger response (e.g., *U. dioica, A. podagraria*). The biomass of an *U. diocia* individual, for instance, was four times higher in post-agricultural versus ancient sites and exceeded the biomass of *P. elatior* individuals with a factor 3.5 (Chapter 4). Growing more vigorously has evident advantages such as a disproportionate benefit with respect to light competition.

1.3 Reproduction and population persistence

Once a forest plant species has formed a (small) founding population in a post-agricultural site, it needs to reproduce to persist and eventually expand. Many may do so through clonal expansion (e.g., *A. nemorosa, C. lutetiana*), but others mainly need to produce offspring that germinated from seeds and recruited into the adult life stage (e.g., *P. elatior, G. urbanum*, *H. non-scripta*). Those new recruits obviously face the same establishment limitations that were outlined above. In our introduction experiments, *G. urbanum* successfully produced new recruits in post-agricultural forest, while *P. elatior* largely lacked reproduction (Chapter 2 and 3). The low recruitment of *P. elatior* comes on top of its reduced longevity at post-agricultural sites, i.e., the species fails to counterbalance mortality and the founding populations are unlikely to persist. A modelling study by Jacquemyn & Brys (2008) similarly showed that populations of *P. elatior* may be in decline in very recent afforestations (< 50 years) because of low seedling and juvenile growth coupled with a low juvenile and adult survival.

Based on the discussion in the previous sections (§1.1 and §1.2), it seems straightforward to attribute the low reproduction of forest plants in post-agricultural sites to the unfavourable impact of interspecific competition on new recruits. Yet, a low seed quantity and quality could also play a role. Our germination experiments (Chapter 6) were among the first to test the effect of former land use on the production of germinable seeds. The results demonstrated that the low recruitment in post-agricultural forest is not caused by limited seed quantity and quality. In fact, the germination percentage even tended to be higher for seeds collected from individuals growing in post-agricultural compared to ancient sites (see also Endels et al. 2004). P availability partly accounted for the land-use effect. While some morphological measures of reproductive performance (e.g., length and number of inflorescences) were unaffected by P (Chapter 5), the germination percentage of *P. elatior* significantly increased with P supply. These observations indicate that the availability of germinable seeds does not cause the deficient reproduction in post-agricultural forests.

1.4 Species-specific bottlenecks for establishment

Three generalities emerged from the summary of our studies that determined land-use effects on forest plant establishment. First, we experimentally demonstrated that environmental (soil) legacies of former agricultural land use may have an important impact on the establishment of forest plants. The leading proposition that dispersal limitation principally controls forest plant colonization into post-agricultural forests therefore seems too restrictive. Obviously, both dispersal and establishment may shape forest understorey plant colonization (cf. Eriksson & Ehrlén 1992; Verheyen & Hermy 2001b; Clark et al. 2007).

Second, it requires only one reduced stage of plant establishment in post-agricultural forest to limit the colonization success of forest plants, irrespective of the impact of former land use on the other stages. The establishment of *P. elatior*, for instance, was reduced by low seedling recruitment and low adult survival while, at the same time, its growth and the production of germinable seeds benefited from the past agricultural land use. Establishment limitation may thus take the form of bottlenecks rather than reflecting an overall reduced establishment in post-agricultural versus ancient forest. Studies focussing on one or several specific establishment stages that found no detrimental impact of past land use cannot not rule out that colonization is impeded at another stage.

Third, the particular establishment stage(s) that limit plant colonization may differ among species because individual life histories cause species to respond differently to the legacies of former agricultural land use. While *P. elatior*, for instance, increased its adult performance in post-agricultural compared to ancient forest or with increased P availability, *A. nemorosa* tended to show the opposite response. On the other hand, the competition with the established vegetation reduced the recruitment of *P. elatior*, but not *A. nemorosa*. In sum, seeing the species-specific causes and consequences of reduced establishment, it seems impossible to make sound generalizations of establishment limitation across species at the level of the individual establishment stages.

2 Synthetic framework for plant community recovery in post-agricultural forests

The resurvey of historical vegetation data after 30 years (Chapter 7) offered a long-term perspective on the community development in forests growing on former agricultural land. Together with our experiments that identified the bottlenecks of plant establishment (§1), this enables us to present a framework for the recovery of the understorey in post-agricultural forests.

Under the assumption that the community assembly is purely dispersal driven, the occurrence of slow colonizers should gradually increase over time (e.g., Jacquemyn et al. 2001; Flinn & Marks 2004; Brunet 2007), which should decrease the compositional differences between ancient and post-agricultural sites. We found no recovery after three decades of spontaneous development, so the community assembly might be additionally controlled by factors other than dispersal. The restricted establishment of forest plants in a dense resident vegetation of competitive species, which we found in the different experimental studies, is probably an important factor. The abundance of particular forest plant species with a competitive component in their strategy increased remarkably over time (e.g., *Rubus fruticosus* agg., *Ribes rubrum* L.*, U. dioica, A. podagraria*). These species share a high-nutrient strategy with rapid growth rates, high biomass production and fast lateral spread (Grime 2001; Craine 2009), which enables them to outcompete recruits of colonizing species.

Furthermore, as the proportion of seeds that effectively recruited into the adult stage was awfully low, the establishment of a small founding population hinges upon a high seed pressure. Because populations of slowly colonizing species in ancient forest sites appear to decline relatively rapidly (Chapter 7), seed sources are progressively depleted and propagule pressure is further reduced. The reduced availability of seed sources in the landscape coupled with the low dispersal capacity, the low probability of recruitment into the adult stage and the low adult survival ultimately lead to the exceptionally slow recovery of forest understorey communities. This outcome proposes a wider perspective on the community recovery in forests on former agricultural land and nuances the leading proposition that dispersal mainly controls species colonization (cf. Verheyen et al. 2003b).

Community development in post-agricultural forests may be synthesized using the framework of plant community assembly in old fields by Cramer et al. (2008) (Fig. 8.1). The framework departs from the degree to which the former agricultural land use has modified the environment (‘Ecosystem state’), i.e., whether or not a biotic and abiotic threshold were crossed. The community development following the cessation of small-scale, non-intensive agriculture (e.g., sowing crops in temporary forest gaps) will follow a broadly repeatable trajectory towards the pre-disturbed state (Fig. 8.1 A). Many forest plants will still occur in relict populations or in the seed bank and the environmental conditions did not change much. It is, however, hard to find evidence for such a scenario. When the biotic threshold is crossed, the agricultural use has removed the local forest plant populations and impoverished the seed bank. In this case, limited dispersal slows down the recovery and shapes the community development along a delayed or novel trajectory with an overrepresentation of good dispersers with high establishment rates (Fig. 8.1 B; Vellend et al. 2007). Although such a scenario represents forests growing on extensively cultivated land (i.e., no strong and persistent abiotic legacies), the recovery may still be delayed for several decades to centuries (e.g., Motzkin et al. 1996; Bellemare et al. 2002; Sciama et al. 2009). Finally, if the past agriculture has pushed a site across the biotic and abiotic threshold, the recovery after abandonment may be stuck in a persistently degraded state (Fig. 8.1 C). Limited dispersal initially constrains plant (re)colonization and the novel environmental conditions (e.g., increased soil nutrients) favour the establishment and growth of competitive species (Cramer et al. 2008). The results from the present thesis provide some evidence that this scenario might apply to forests growing on former agricultural land that has been heavily fertilized.

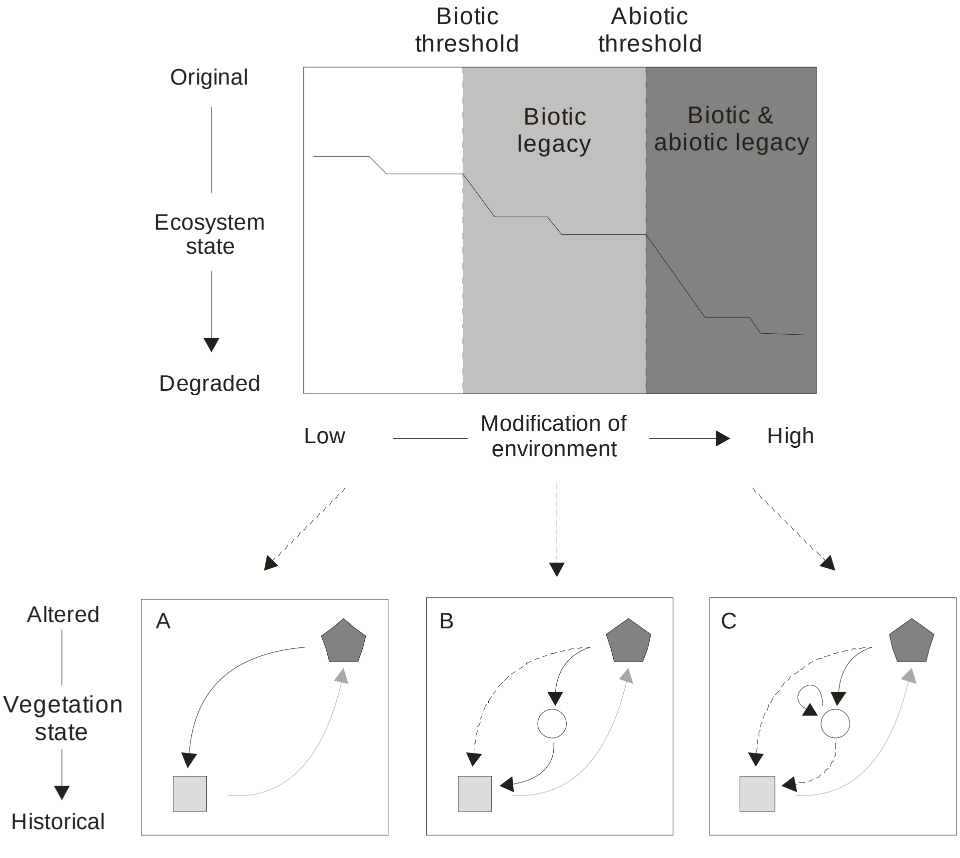


Fig. 8.1 Synthetic framework presenting three scenarios of community development in post-agricultural forests (adapted from Cramer et al. (2008)). The degree to which the ecosystem is degraded by the past agriculture depends on the legacies of the agricultural use (no legacy, biotic legacy, biotic and abiotic legacy) and is related to the trajectory of the subsequent vegetation development. If the agricultural use left almost no persistent legacies, the development of understorey plant communities may follow a broadly repeatable trajectory (A). Biotic legacies lead to a delayed or novel trajectory (B). In case of biotic and abiotic legacies, the vegetation development may be stuck in a persistently degraded state (C). A comprehensive explanation of the scenarios with examples can be found in the text.

In addition, it is important to notice that the trajectories of community development outlined above are likely to be influenced by environmental changes other than those related to land-use change that occur simultaneously. High levels of N deposition will, for instance, most probably have an effect (e.g. Gilliam 2006). Even if the former land use has not left strong abiotic legacies (scenario B), community development may still persist in a degraded state because of limitations related to the adverse impact of N deposition on forest communities. It may be possible that particular forest species are (just) able to persist under the prevailing N deposition levels in ancient forest, but are not capable of expanding and successfully colonizing to newly established sites (cf. Hautier et al. 2009).

3 Management consequences

Facilitating the colonization of forest plants and accelerating the community development in post-agricultural forests requires management strategies that tackle both dispersal and establishment limitation. The relaxation of dispersal constraints basically involves landscape-level actions. A strategic planning and deliberate realization of forest expansion is a prerequisite for individuals to disperse to the new forests. Honnay et al. (2002a) argue in favour of new forest establishment adjacent to ancient forest patches, but the proportion of ancient forest patches in the landscape that support source populations may also be crucial (Vellend 2003). The latter suggests to concentrate management efforts on landscapes that are not completely impoverished, in which ancient forest patches with source populations do occur. A more direct, but highly challenged intervention strategy would be to introduce seeds or adults of particularly slowly colonizing species into post-agricultural forests. The potential benefits and risks of such ‘managed relocation’ (also referred to as ‘assisted colonization’ or ‘assisted migration’) are a topical subject in the parlance of the current debate on climate-driven extinctions (McLachlan et al. 2007; Richardson et al. 2009). It might be useful to extend this debate within the context of impoverished post-agricultural landscapes. At present, the majority of the slow colonizers are not at immediate risk of extinction (e.g., not on the red list), so hasty management actions should be avoided.

The low establishment rates should be handled at the local scale. The present thesis provided evidence that the soil nutrient legacy of past agriculture, and P in particular, may impede the recruitment and survival of forest plant species. Lowering nutrient availability before afforestation could therefore be considered. Active restoration measures that have been applied to other vegetation types (e.g., species-rich grassland, wetland vegetation and heathland) include frequent hay cutting, grazing, liming, deep cultivation and topsoil removal (e.g., Pywell et al. 2002; Sival et al. 2004; Klimkowska et al. 2007). In already established forest or when lowering the nutrient levels before afforestion is not regarded feasible (e.g., costly, large area, fast compensation for deforestation elsewhere is needed, focus on stand productivity), mitigating the impact of the soil nutrient legacies is required. One way of doing this is keeping the light levels at the forest floor low (e.g., promoting a dense shrub layer) to reduce the competitive dominance of vigorous species through light limitation (De Keersmaeker et al. 2004).

An important drawback of the light limitation strategy is, of course, that the low light availability also affects the performance of target species. Low light levels might significantly reduce the reproductive performance of forest plants (Chapter 5) and may suppress sexual reproduction and trigger clonal growth (Verburg & Grava 1998; Honnay & Bossuyt 2005). Prolonged clonal growth may ultimately lead to monoclonal populations with reduced viability (Honnay & Bossuyt 2005). Furthermore, the species pool of forests in much of western Europe still bears the ecological memory of past traditional forest management regimes such as coppice, coppice-with-standards and livestock grazing (Peterken 1993; Van Calster 2008). Those regimes created relatively open forests characterized by periods of high light availability. Numerous plant species of half-open habitats thrived in those forest habitats (e.g., *Cardamine pratensis* L.*, Circium palustre* (L.) Scop.*, Primula vulgaris* Huds.) and forests may actually serve as a refuge for non-woodland species that are eliminated from the surrounding intensive landscape (Peterken & Francis 1999). Therefore, a good management option would be to promote a dense shrub layer that suppresses the competitive species and to cut this layer periodically. The regular cutting promotes the generative reproduction, which stimulates the colonization of forest plants (De Keersmaeker et al. 2010), and allows heliophilous non-woodland species to persist in the seed bank and reappear at each cutting (Brown & Oosterhuis 1981; Van Calster et al. 2008b; Chapter 8 in Plue 2010).

4 Suggestions for further research

Further research should involve both fundamental studies that elaborate on particular mechanisms of establishment limitation and applied studies that aim at making current knowledge more operational for managers and decision makers. A number of suggestions were already made in the respective chapters, but some of these are highlighted here. Further fundamental research is needed to uncover the mechanisms that underlie the deleterious effect of interspecific competition on plant recruitment and survival in post-agricultural forests. More specifically, competition for light is expected to be a major driver of species exclusion in nutrient-rich environments (Craine 2009), but its importance for establishment limitation in post-agricultural forests needs additional research. In experimental grassland communities, Hautier et al. (2009) showed that transplanted seedling mortality was much higher in fertilized plots versus controls, but not if light was added below the canopy layer of tall-growing dominant species. Similar experiments for forest communities would be an important step forward.

Similar to most previous studies, we have mainly focused on the soil nutrient legacy of past agriculture that affects the colonizing forest species both directly and indirectly, e.g., via competitive interactions with the established vegetation. Yet, the role of other interactions involving plant mutualists and enemies is still unclear (Flinn & Vellend 2005). One of the appealing new directions is the link between the underground community and the performance of forest plants. Performance differences may, for instance, arise if the distribution of mycorrhizal fungi with a high degree of specificity towards particular host forest plants – offering the greatest symbiotic benefit to those species – is also controlled by land-use history (cf. Helgason et al. 2007). The same applies if the different mycorrhizal communities in ancient and post-agricultural sites are also functionally distinct, i.e., providing different benefits to the host plants (Fitter 2005). This topic might be further extended to multitrophic interactions.

Finally, it is advisable that applied research translates the current knowledge about land-use effects on the understorey diversity into practical guidelines for future afforestations of agricultural land and the management of recent afforestations. This includes defining spatial criteria for afforestation sites that account for the distribution of source populations in the landscape, determining threshold nutrient levels for favourable understorey community development, assessing tree species effects on the soil, litter and vegetation development and evaluating different cutting regimes of the tree and shrub layer. Ultimately, these guidelines should result in the selection and management of sites with high understorey recovery potential. Site selection should, however, also account for ecosystem attributes and services other than biodiversity, e.g., site productivity and nutrient cycling. The development or extension (e.g., AFFOREST–sDSS, Gilliams et al. 2004) of decision support systems that include a multitude of ecosystem attributes and that are spatially explicit would be useful for the future planning of afforestations.

REFERENCES

Aber JD, Nadelhoffer KJ, Steudler P, Melillo JM (1989) Nitrogen saturation in northern forest ecosystems. Bioscience 39, 378-386.

Aerts R, Chapin FS (2000) The mineral nutrition of wild plants revisited: A re-evaluation of processes and patterns. Advances in Ecological Research 30, 1-67.

Al-Mufti MM, Sydes CL, Furness SB, Grime JP, Band SR (1977) Quantitative analysis of shoot phenology and dominance in herbaceous vegetation. Journal of Ecology 65, 759-791.

Allison V J (2002) Nutrients, arbuscular mycorrhizas and competition interact to influence seed production and germination success in *Achillea millefolium*. Functional Ecology 16, 742-749.

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecology 26, 32-46.

Anderson WB (2003) Interactions of nutrient effects with other biotic factors in the herbaceous layer. In: Gilliam FS, Roberts MR (eds) The herbaceous layer in forests of eastern North America. Oxford University Press, Oxford, UK.

Baeté H, De Bie M, Hermy M, Van den Bremt P (2009) Miradal. Erfgoed in Heverleebos en Meerdaalwoud. [Miradal. Heritage in Heverlee forest and Meerdaal forest] (in Dutch) Davidsfonds, Leuven, Belgium.

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

Baeten L, De Frenne P, Verheyen K, Graae BJ, Hermy M (2010) Forest herbs in the face of global change: a single-species-multiple-threats approach for *Anemone nemorosa*. Plant Ecology and Evolution 143, 19-30.

Bates D, Maechler M (2010) lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-33. [URL http://CRAN.R-project.org/package=lme4]

Bellemare J, Motzkin G, Foster DR (2002) Legacies of the agricultural past in the forested present: an assessment of historical land-use effects on rich mesic forests. Journal of Biogeography 29, 1401-1420.

Bossuyt B, Deckers J, Hermy M (1999a) A field methodology for assessing man-made disturbance in forest soils developed in loess. Soil Use and Management 15, 14-20.

Bossuyt B, Hermy M, Deckers J (1999b) Migration of herbaceous plant species across ancient-recent forest ecotones in central Belgium. Journal of Ecology 87, 628-638.

Bradshaw AD (1965) Evolutionary significance of phenotypic plasticity in plants. Advances in Genetics 13, 115-155.

Brewer R (1980) A half-century of changes in the herb layer of a climax deciduous forest in Michigan. Journal of Ecology 68, 823-832.

Brown AHF, Oosterhuis L (1981) The role of buried seed in coppicewoods. Biological Conservation 21, 19-38.

Brunet J (2007) Plant colonization in heterogeneous landscapes: an 80-year perspective on restoration of broadleaved forest vegetation. Journal of Applied Ecology 44, 563-572.

Brunet J, Von Oheimb G (1998) Migration of vascular plants to secondary woodlands in southern Sweden. Journal of Ecology 86, 429-438.

Callaway RM, Pennings SC, Richards CL (2003) Phenotypic plasticity and interactions among plants. Ecology 84, 1115-1128.

Chase JM, Leibold MA (2003) Ecological niches. Linking classical and contemporary approaches. The University of Chicago Press, Chicago, USA.

Clark JS, Macklin E, Wood L (1998) Stages and spatial scales of recruitment limitation in southern Appalachian forests. Ecological Monographs 68, 213-235.

Clark CJ, Poulsen JR, Levey DJ, Osenberg CW (2007) Are plant populations seed limited? A critique and meta-analysis of seed addition experiments. American Naturalist 170, 128-142.

Compton JE, Boone RD (2000) Long-term impacts of agriculture on soil carbon and nitrogen in New England forests. Ecology 81, 2314-2330.

Compton JE, Boone RD, Motzkin G, Foster DR (1998) Soil carbon and nitrogen in a pine-oak sand plain in central Massachusetts: Role of vegetation and land-use history. Oecologia 116, 536-542.

Craine JM (2005) Reconciling plant strategy theories of Grime and Tilman. Journal of Ecology 93, 1041-1052.

Craine JM (2009) Resource strategies of wild plants. Princeton University Press, Princeton, USA.

Cramer VA, Hobbs RJ, Standish RJ (2008) What's new about old fields? Land abandonment and ecosystem assembly. Trends in Ecology and Evolution 23, 104-112.

Crawley MJ (2003) The structure of plant communities. In: Crawley MJ (ed) Plant ecology. Blackwell Science, Oxford, UK.

Dambrine E, Dupouey JL, Laut L, Humbert L, Thinon M, Beaufils T, Richard H (2007) Present forest biodiversity patterns in France related to former Roman agriculture. Ecology 88, 1430-1439.

Davidson EA, Ackerman IL (1993) Changes in soil carbon inventories following cultivation of previously untilled soils. Biogeochemistry 20, 161-193.

De Keersmaeker L, Rogiers N, Lauriks R, Esprit M, Vanhoutte L (2001) Ecosysteemvisie bos Vlaanderen: ruimtelijke uitwerking van de natuurlijke bostypes op basis van bodemgroeperingseenheden en historische boskaarten. [Ecosystem concept for forests in Flanders: spatial elaboration of the natural forest types based on soil classes and historical forest maps] (in Dutch) IBW, Geraardsbergen, Belgium.

De Keersmaeker L, Martens L, Verheyen K, Hermy M, De Schrijver A, Lust N (2004) Impact of soil fertility and insolation on diversity of herbaceous woodland species colonizing afforestations in Muizen Forest (Belgium). Forest Ecology and Management 188, 291-304.

De Keersmaeker L, Vandekerkhove K, Verstraeten A, Baeten L, Verschelde P, Thomaes A, Hermy M, Verheyen K (2010) Clear-felling effects on colonization rates of shade-tolerant forest herbs into a post-agricultural forest adjacent to ancient forest. Applied Vegetation Science, in press. doi 10.1111/j.1654-109X.2010.01101.x

DeWitt TJ, Sih A, Wilson DS (1998) Costs and limits of phenotypic plasticity. Trends in Ecology & Evolution 13, 77-81.

Donohue K, Foster DR, Motzkin G (2000) Effects of the past and the present on species distribution: land-use history and demography of wintergreen. Journal of Ecology 88, 303-316.

Donohue K, Pyle EH, Messiqua D, Heschel MS, Schmitt J (2001) Adaptive divergence in plasticity in natural populations of *Impatiens capensis* and its consequences for performance in novel habitats. Evolution 55, 692-702.

Duffy D-C, Meier A-J (1992) Do Appalachian herbaceous understories ever recover from clearcutting? Conservation Biology 6, 196-201.

Dupouey JL, Dambrine E, Laffite JD, Moares C (2002) Irreversible impact of past land use on forest soils and biodiversity. Ecology 83, 2978-2984.

Dzwonko Z, Loster S (1989) Distribution of vascular plant species in small woodlands on the western Carpathian foothills. Oikos 56, 77-86.

Ehrlén J, Eriksson O (2000) Dispersal limitation and patch occupancy in forest herbs. Ecology 81, 1667-1674.

Ehrlén J, Lehtilä K (2002) How perennial are perennial plants? Oikos 98, 308-322.

Ehrlén J, Munzbergova Z, Diekmann M, Eriksson O (2006) Long-term assessment of seed limitation in plants: results from an 11-year experiment. Journal of Ecology 94, 1224-1232.

Eickmeier WG, Schussler EE (1993) Responses of the spring ephemeral *Claytonia virginica* L to light and nutrient manipulations and implications for the Vernal-Dam Hypothesis. Bulletin of the Torrey Botanical Club 120, 157-165.

Elemans M (2004) Light, nutrients and the growth of herbaceous forest species. Acta Oecologica–International Journal of Ecology 26, 197-202.

Endels P, Adriaens D, Verheyen K, Hermy M (2004) Population structure and adult plant performance of forest herbs in three contrasting habitats. Ecography 27, 225-241.

Eriksson O, Ehrlén J (1992) Seed and microsite limitation of recruitment in plant-populations. Oecologia 91, 360-364.

Falkengren-Grerup U, ten Brink DJ, Brunet J (2006) Land use effects on soil N, P, C and pH persist over 40-80 years of forest growth on agricultural soils. Forest Ecology and Management 225, 74-81.

Fitter AH (2005) Darkness visible: reflections on underground ecology. Journal of Ecology 93, 231-243.

Fitter AH, Peat HJ (1994) The ecological flora database. Journal of Ecology 82, 415-425. [URL http://www.ecoflora.co.uk, accessed May 2008]

Flinn KM (2007) Microsite-limited recruitment controls fern colonization of post-agricultural forests. Ecology 88, 3103-114.

Flinn KM, Marks PL (2004) Land-use history and forest herb diversity in Tompkins county. In: Honnay O, verheyen K, Bossuyt B, Hermy M (eds) Forest biodiversity. Lessons from history for conservation. CABI Publishing, Wallingford, UK.

Flinn KM, Marks PL (2007) Agricultural legacies in forest environments: Tree communities, soil properties, and light availability. Ecological Applications 17, 452-463.

Flinn KM, Vellend M (2005) Recovery of forest plant communities in post-agricultural landscapes. Frontiers in Ecology and the Environment 3, 243-250.

Flinn KM, Vellend M, Marks PL (2005) Environmental causes and consequences of forest clearance and agricultural abandonment in central New York, USA. Journal of Biogeography 32, 439-452.

Foster DR, Motzkin G, Slater B (1998) Land-use history as long-term broad-scale disturbance: regional forest dynamics in central New England. Ecosystems 1, 96-119.

FRA (2005) The Global Forest Resource Assessment. Progress towards sustainable forest management. Food and Agriculture Organization of the United Nations, Rome, Italy.

Fraterrigo JM, Turner MG, Pearson SM, Dixon P (2005) Effects of past land use on spatial heterogeneity of soil nutrients in southern Appalachian forests. Ecological Monographs 75, 215-230.

Fraterrigo JM, Turner MG, Pearson SM (2006) Previous land use alters plant allocation and growth in forest herbs. Journal of Ecology 94, 548-557.

Fraterrigo JM, Pearson SM, Turner MG (2009) The response of understory herbaceous plants to nitrogen fertilization in forests of different land-use history. Forest Ecology and Management 257, 2182-2188.

Froment A, Tanghe M (1967) Répercussion des formes anciennes d'agriculture sur les sols et la composition floristique. [Consequences of former agriculture on soils and the floristic composition] (in French) Bulletin de la Société Royale Botanique de Belgique 100, 335-351.

Gilliam FS (2006) Response of the herbaceous layer of forest ecosystems to excess nitrogen deposition. Journal of Ecology 94, 1176-1191.

Gilliam FS (2007) The ecological significance of the herbaceous layer in temperate forest ecosystems. Bioscience 57, 845-858.

Gilliams S, Van Orshoven J, Hansen K, Skov-Petersen H, Bart M (2004) The AFFOREST-sDSS tutorial, K.U.Leuven, Leuven, Belgium. [URL http://www.sl.kvl.dk/afforest/, accessed September 2010]

Graae BJ (2000) The effect of landscape fragmentation on forest floor species in two regions of Denmark, Hornsherred and Himmerland. Journal of Vegetation Science 11, 881-892.

Graae BJ, Sunde PB (2000) The impact of forest continuity and management on forest floor vegetation evaluated by species traits. Ecography 23, 720-731.

Graae BJ, Sunde PB, Fritzboger B (2003) Vegetation and soil differences in ancient opposed to new forests. Forest Ecology and Management 177, 179-190.

Graae BJ, Hansen T, Sunde PB (2004) The importance of recruitment limitation in forest plant species colonization: a seed sowing experiment. Flora 199, 263-270.

Graae BJ, Verheyen K, Kolb A, Van Der Veken S, et al. (2009) Germination requirements and seed mass of slow- and fast-colonizing temperate forest herbs along a latitudinal gradient. Ecoscience 16, 248-257.

Grashof-Bokdam CJ, Geertsema W (1998) The effect of isolation and history on colonization patterns of plant species in secondary woodland. Journal of Biogeography 25, 837-846.

Gress SE, Nichols TD, Northcraft CC, Peterjohn WT (2007) Nutrient limitation in soils exhibiting differing nitrogen availabilities: What lies beyond nitrogen saturation? Ecology 88, 119-130.

Griffith TM, Sultan SE (2005) Shade tolerance plasticity in response to neutral vs green shade cues in *Polygonum* species of contrasting ecological breadth. New Phytologist 166, 141-147.

Grime JP (2001) Plant strategies, vegetation processes, and ecosystem properties. John Wiley & Sons, Chichester, UK.

Grime JP, Thompson K, Hunt R, Hodgson JG, et al. (1997) Integrated screening validates primary axes of specialisation in plants. Oikos 79, 259-281.

Gundersen P, Schmidt IK, Raulund-Rasmussen K (2006) Leaching of nitrate from temperate forests – effects of air pollution and forest management. Environmental Reviews 14, 1-57.

Güsewell S (2004) N:P ratios in terrestrial plants: variation and functional significance. New Phytologist 164, 243-266.

Güsewell S, Gessner MO (2009) N:P ratios influence litter decomposition and colonization by fungi and bacteria in microcosms. Functional Ecology 23, 211-219.

Gustafsson C, Ehrlén J, Eriksson O (2002) Recruitment in *Dentaria bulbifera*; the roles of dispersal, habitat quality and mollusc herbivory. Journal of Vegetation Science 13, 719-724.

Hall B, Motzkin G, Foster DR, Syfert M, Burk J (2002) Three hundred years of forest and land-use change in Massachusetts, USA. Journal of Biogeography 29, 1319-1335.

Hansen K, Schmidt IK, Gundersen P, Ritter E (2002) Effect of afforestation on soil N, nitrate leaching, and pH. In: Hansen K (ed) Planning afforestation on previously managed arable land – influence on deposition, nitrate leaching, precipitation surplus, and carbon sequestration. Litterature review AFFOREST project. Forest & Landscape, Horsholm, Denmark.

Hansen K, Rosenqvist L, Vesterdal L, Gundersen P (2007) Nitrate leaching from three afforestation chronosequences on former arable land in Denmark. Global Change Biology 13, 1250-1264.

Hanski I (2000) Extinction debt and species credit in boreal forests: modelling the consequences of different approaches to biodiversity conservation. Annales Zoologici Fennici 37, 271-280.

Harper JL (1977) Population biology of plants. Academic Press, London, UK.

Harpole WS, Tilman D (2007) Grassland species loss resulting from reduced niche dimension. Nature 446, 791-793.

Hautier Y, Niklaus PA, Hector A (2009) Competition for light causes plant biodiversity loss after eutrophication. Science 324, 636-638.

Heddle EM, Specht RL (1975) Dark Island heath (90-Mile Plain, south-Australia). 8. Effect of fertilizers on composition and growth, 1950-1972. Australian Journal of Botany 23, 151-164.

Helgason T, Merryweather JW, Young JPW, Fitter AH (2007) Specificity and resilience in the arbuscular mycorrhizal fungi of a natural woodland community. Journal of Ecology 95, 623-630.

Hermy M (1985) Ecologie en fytosociologie van oude en jonge bossen in binnen-Vlaanderen. [Ecology and phytosociology of ancient and recent forests in the county of Flanders] (in Dutch) PhD thesis, Ghent University, Ghent, Belgium.

Hermy M, Stieperaere H (1981) An indirect gradient analysis of the ecological relationships between ancient and recent riverine woodlands to the south of Bruges (Flanders, Belgium). Vegetatio 44, 43-49.

Hermy M, Verheyen K (2007) Legacies of the past in the present-day forest biodiversity: a review of past land-use effects on forest plant species composition and diversity. Ecological Research 22, 361-371.

Hermy M, van den Bremt P, Tack G (1993) Effects of site history on woodland vegetation. In: Broekmeyer MEA, Vos W, Koop H (eds) European forest reserves. Pudoc, Wageningen, the Netherlands.

Hermy M, Honnay O, Firbank L, Grashof-Bokdam C, Lawesson JE (1999) An ecological comparison between ancient and other forest plant species of Europe, and the implications for forest conservation. Biological Conservation 91, 9-22.

Hilborn R, Mangel M (1997) The ecological detective: confronting models with data. Princeton University Press, Princeton, USA.

Hipps NA, Davies MJ, Dodds P, Buckley GP (2005) The effects of phosphorus nutrition and soil pH on the growth of some ancient woodland indicator plants and their interaction with competitor species. Plant and Soil 271, 131-141.

Honnay O, Bossuyt B (2005) Prolonged clonal growth: escape route or route to extinction? Oikos 108, 427-432.

Honnay O, Degroote B, Hermy M (1998) Ancient-forest plant species in western Belgium: A species list and possible ecological mechanisms. Belgian Journal of Botany 130, 139-154.

Honnay O, Hermy M, Coppin P (1999) Impact of habitat quality on forest plant species colonization. Forest Ecology and Management 115, 157-170.

Honnay O, Bossuyt B, Verheyen K, Butaye J, Jacquemyn H, Hermy M (2002a) Ecological perspectives for the restoration of plant communities in European temperate forests. Biodiversity and Conservation 11, 213-242.

Honnay O, Verheyen K, Butaye J, Jacquemyn H, Bossuyt B, Hermy M (2002b) Possible effects of habitat fragmentation and climate change on the range of forest plant species. Ecology Letters 5, 525-530.

Honnay O, Verheyen K, Bossuyt B, Hermy M (2004) Forest biodiversity. Lessons from history for conservation. CABI Publishing, Wallingford, UK.

Honnay O, Jacquemyn H, Bossuyt B, Hermy M (2005) Forest fragmentation effects on patch occupancy and population viability of herbaceous plant species. New Phytologist 166, 723-736.

Hooker TD, Compton JE (2003) Forest ecosystem carbon and nitrogen accumulation during the first century after agricultural abandonment. Ecological Applications 13, 299-313.

Hubbell SP (2001) The unified neutral theory of biodiversity and biogeography. Princeton University Press, Princeton, USA.

Hunt R, Hodgson JG, Thompson K, Bungener P, Dunnett NP, Askew AP (2004) A new practical tool for deriving a functional signature for herbaceous vegetation. Applied Vegetation Science 7, 163-170.

Jabiol B, Brêthes A, Ponge JE, Toutain F, Brun J-J (1995) L' humus sous toutes ses formes. [Humus and its different forms] (in French) ENGREF, Nancy, France.

Jackson ST, Sax DF (2009) Balancing biodiversity in a changing environment: extinction debt, immigration credit and species turnover. Trends in Ecology & Evolution 25, 153-160.

Jacquemyn H, Brys R (2008) Effects of stand age on the demography of a temperate forest herb in post-agricultural forests. Ecology 89, 3480-3489.

Jacquemyn H, Butaye J, Hermy M (2001) Forest plant species richness in small, fragmented mixed deciduous forest patches: the role of area, time and dispersal limitation. Journal of Biogeography 28, 801-812.

Jacquemyn H, Brys R, Hermy M (2002) Patch occupancy, population size and reproductive success of a forest herb (*Primula elatior*) in a fragmented landscape. Oecologia 130, 617-625.

Jacquemyn H, Vandepitte K, Roldan-Ruiz I, Honnay O (2009) Rapid loss of genetic variation in a founding population of *Primula elatior* (Primulaceae) after colonization. Annals of Botany 103, 777-783.

Kirby JK, Watkins C (1998) The ecological history of European forests. CAB International, New York, USA.

Klimkowska A, Van Diggelen R, Bakker JP, Grootjans AP (2007) Wet meadow restoration in Western Europe: A quantitative assessment of the effectiveness of several techniques. Biological Conservation 140, 318-328.

Koerner W, Dupouey JL, Dambrine E, Benoit M (1997) Influence of past land use on the vegetation and soils of present day forest in the Vosges mountains, France. Journal of Ecology 85, 351-358.

Kopecký M, Vojta J (2009) Land use legacies in post-agricultural forests in the Doupovské Mountains, Czech Republic. Applied Vegetation Science 12, 251-260.

Lameire S, Hermy M, Honnay O (2000) Two decades of change in the ground vegetation of a mixed deciduous forest in an agricultural landscape. Journal of Vegetation Science 11, 695-704.

Lepš J, Šmilauer P (2003) Multivariate analysis of ecological data using CANOCO. Cambridge University Press, Cambridge.

MacArthur RH, Wilson EO (1967) The theory of island biogeography. Princeton University Press, Princeton, USA.

Marschner H (1995) Mineral nutrition of higher plants. Academic Press, London, UK.

Matlack GR (1994) Plant-species migration in a mixed-history forest landscape in eastern North-America. Ecology 75, 1491-1502.

Matlack GR (2009) Long-term changes in soils of second-growth forest following abandonment from agriculture. Journal of Biogeography 36, 2066-2075.

Mcardle BH, Anderson MJ (2001) Fitting multivariate models to community data: a comment on distance-based redundancy analysis. Ecology 82, 290-297.

McLachlan JS, Hellmann JJ, Schwartz MW (2007) A framework for debate of assisted migration in an era of climate change. Conservation Biology 21, 297-302.

McLauchlan K (2006) The nature and longevity of agricultural impacts on soil carbon and nutrients: A review. Ecosystems 9, 1364-1382.

Meekins JF, McCarthy BC (2000) Responses of the biennial forest herb *Alliaria petiolata* to variation in population density, nutrient addition and light availability. Journal of Ecology 88, 447-463.

Mina-plan 3 (2003) Milieubeleidsplan 2003-2007. [Environmental policy plan 2003-2007] (in Dutch) Ministerie van de Vlaamse Gemeenschap, Brussel, Belgium.

Miner BG, Sultan SE, Morgan SG, Padilla DK, Relyea RA (2005) Ecological consequences of phenotypic plasticity. Trends in Ecology & Evolution 20, 685-692.

Motzkin G, Foster D, Allen A, Harrod J, Boone R (1996) Controlling site to evaluate history: vegetation patterns of a New England sand plain. Ecological Monographs 66, 345-365.

Muller RN (2003) Nutrient relations of the herbaceous layer in deciduous forest ecosystems. In: Gilliam FS, Roberts MR (eds) The herbaceous layer in forests of eastern North America. Oxford University Press, Oxford, UK.

Natuurindicatoren (2006) Natuurindicatoren. Cijfers voor het beleid. [Nature indicators. Figures for policy makers] (in Dutch) URL www.natuurindicatoren.be, accessed August 2010.

Neirynck J, Genouw G, Coenen S, Roskams P (2004) Depositie en luchtkwaliteit in Vlaamse bossgebieden. [Deposition and air quality in Flemish forest areas] (in Dutch) Instituut voor Bosbouw en Wildbeheer, Geraardsbergen, Belgium.

Neter J, Kutner MH, Nachtsheim CJ, Wasserman W (1996) Applied linear statistical models. McGraw-Hill, Boston, USA.

Neufield HS, Young DR (2003) Ecophysiology of the herbaceous layer in temperate deciduous forests. In: Gilliam FS, Roberts MR (eds) The herbaceous layer in forests of eastern North America. Oxford University Press, Oxford, UK.

Noirfalise A (1984) Forêts et stations forestières en Belgique. [Forests and forest stands in Belgium] (in French) Press Agronomique, Gembloux, Belgium.

Olden JD, Rooney TP (2006) On defining and quantifying biotic homogenization. Global Ecology and Biogeography 15, 113-120.

Orczewska A (2009) Migration of herbaceous woodland flora into post-agricultural black alder woods planted on wet and fertile habitats in south western Poland. Plant Ecology 204, 83-96.

Packham JR (1978) Biological flora of British isles - *Oxalis-Acetosella* L. Journal of Ecology 66, 669-693.

Peñuelas J (2005) Plant physiology – a big issue for trees. Nature 437, 965-966.

Peterken GF (1974) A method for assessing woodland flora for conservation using indicator species. Biological Conservation 6, 239-245.

Peterken GF (1993) Woodland conservation and management. Chapman & Hall, London, UK.

Peterken GF (1996) Natural woodland: ecology and conservation in northern temperate regions. Cambridge University Press, Cambridge, UK.

Peterken GF, Francis JL (1999) Open spaces as habitats for vascular ground flora species in the woods of central Lincolnshire, UK. Biological Conservation 91, 55-72.

Peterken GF, Game M (1984) Historical factors affecting the number and distribution of vascular plant species in the woodlands of central Lincolnshire. The Journal of Ecology 72, 155-182.

Petersen PM (1994) Flora, vegetation, and soil in broadleaved ancient and planted woodland, and scrub on Røsnæs, Denmark. Nordic Journal of Botany 14, 693-709.

Picket STA, White PS (1985) The ecology of natural disturbance and patch dynamics. Academic Press, Orlando, USA.

Pigott CD (1971) Analysis of the response of *Urtica dioica* to phosphate. New Phytologist 70, 935-966.

Pigott CD (1982) The experimental study of vegetation. New Phytologist 90, 389-404.

Pigott CD, Taylor K (1964) The distribution of some woodland herbs in relation to the supply of nitrogen and phosphorus in the soil. Journal of Ecology 52, 175-185.

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2009) nlme: linear and nonlinear mixed effects models. R package version 3.1-96.

Plue J (2010) Impact of land use change and forest management on the soil seed bank of deciduous forests. PhD thesis, K.U.Leuven, Leuven, Belgium.

Plue J, Hermy M, Verheyen K, Thuillier P, Saguez R, Decocq G (2008) Persistent changes in forest vegetation and seed bank 1,600 years after human occupation. Landscape Ecology 23, 673-688.

Pons TL (1977) Ecophysiological study in field layer of ash coppice. 3. Influence of Diminishing light-intensity during growth on *Geum urbanum* and *Cirsium palustris*. Acta Botanica Neerlandica 26, 251-263.

Pywell RF, Bullock JM, Hopkins A, Walker KJ, Sparks TH, Burke MJW, Peel S (2002) Restoration of species-rich grassland on arable land: assessing the limiting processes using a multi-site experiment. Journal of Applied Ecology 39, 294-309.

R Development Core Team (2009) R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. [URL http://www.R-project.org]

Rackham O (2003) Ancient woodland. Its history, vegetation and uses in England. Castlepoint press, Kirkcudbrightshire, UK.

Rackham O (2008) Ancient woodlands: modern threats. New Phytologist 180, 571-586.

Reader RJ (1993) Control of seedling emergence by ground cover and seed predation in relation to seed size for some old-field species. Journal of Ecology 81, 169-175.

Richardson DM, Hellmann JJ, McLachlan JS, Sax DF, et al. (2009) Multidimensional evaluation of managed relocation. Proceedings of the National Academy of Sciences of the United States of America 106, 9721-9724.

Richter DD, Markewitz D (2001) Understanding soil change: soil sustainability over millennia, centuries, and decades. Cambridge Universtity Press, Cambridge, UK.

Richter DD, Markewitz D, Wells CG, Allen HL, April R, Heine PR, Urrego B (1994) Soil chemical change during three decades in an old-field loblolly pine (*Pinus taeda* L) ecosystem. Ecology 75, 1463-1473.

Richter DD, Allen HL, Li J, Markewitz D, Raikes J (2006) Bioavailibility of slowly cycling soil phosphorus: major restructuring of soil P fractions over four decades in an aggrading forest. Oecologia 150, 259-271.

Roach, DA & Wulff RD (1987) Maternal effects in plants. Annual Review of Ecology Systematics 18, 209-235.

Rogers DA, Rooney TP, Olson D, Waller DM (2008) Shifts in southern Wisconsin forest canopy and understory richness, composition, and heterogeneity. Ecology 89, 2482-2492.

Rogers DA, Rooney TP, Hawbaker TJ, Radeloff VC, Waller DM (2009) Paying the extinction debt in southern Wisconsin forest understories. Conservation Biology 23, 1497-1506.

Roisin P, Thill A (1953) Aperçu de la végétation forestière de quelques bois de la région Sablo-Limoneuse (District Picardo-brabançon). [Survey of the forest vegetation in a number of forests in the sandy-loamy region] (in French) Bulletin de la Société Royale Forestière de Belgique 59, 5-58.

Rooney TP, Wiegmann SM, Rogers DA, Waller DM (2004) Biotic impoverishment and homogenization in unfragmented forest understory communities. Conservation Biology 18, 787-798.

Rorison IH (1968) Response to phosphorus of some ecologically distinct plant species. I. Growth rates and phosphorus absorption. New Phytologist 67, 913-&.

Scheel KC (1936) Colorimetric determination of phosphoric acid in fertilizers with the Pulfrich photometer. Zeitschrift für Analytische Chemie 105, 256-269.

Schlichting CD (1986) The evolution of phenotypic plasticity in plants. Annual Review of Ecology and Systematics 17, 667-693.

Schulze E-D (2000) Carbon and nitrogen cycling in European forest ecosystems. Springer-Verlag, Berlin, Germany.

Schupp EW (1995) Seed seedling conflicts, habitat choice, and patterns of plant recruitment. American Journal of Botany 82, 399-409.

Sciama D, Augusto L, Dupouey JL, Gonzalez M, Dominguez CM (2009) Floristic and ecological differences between recent and ancient forests growing on non-acidic soils. Forest Ecology and Management 258, 600-608.

Silvertown J, Franco M, Pisanty I, Mendoza A (1993) Comparative plant demography - Relative importance of life-cycle components to the finite rate of increase in woody and herbaceous perennials. Journal of Ecology 81, 465-476.

Silvertown J, Poulton P, Johnston E, Edwards G, Heard M, Biss PM (2006) The Park Grass Experiment 1856-2006: Its contribution to ecology. Journal of Ecology 94, 801-814.

Singleton R, Gardescu S, Marks PL, Geber MA (2001) Forest herb colonization of postagricultural forests in central New York State, USA. Journal of Ecology 89, 325-338.

Sival FP, Chardon WJ, van der Werff MM (2004) Natuurontwikkeling op voormalige landbouwgronden in relatie tot de beschikbaarheid van fosfaat: evaluatie van verschralingsmaatregelen. Alterra raport 951. [Nature development on post-agricultural land and the relation with the availability of phosphate: evalation of measures to reduce nutrient availability] (in Dutch) Alterra, Wageningen, the Netherlands.

Smith WB, Miles PD, Vissage JS, Pugh SA (2004) Forest resources of the United States, 2002. General Technical report NC-241. USDA Forest Service, North central forest experimental station, St. Paul, USA.

Smith MD, Knapp AK, Collins SL (2009) A framework for assessing ecosystem dynamics in response to chronic resource alterations induced by global change. Ecology 90, 3279-3289.

Sultan SE (2000) Phenotypic plasticity for plant development, function and life history. Trends in Plant Science 5, 537-542.

Sultan SE (2001) Phenotypic plasticity for fitness components in *Polygonum* species of contrasting ecological breadth. Ecology 82, 328-343.

Sydes C, Grime JP (1981) Effects of tree leaf litter on herbaceous vegetation in deciduous woodland. 1. Field investigations. Journal of Ecology 69, 237-248.

Tack G, Van Den Bremt P, Hermy M (1993) Bossen van Vlaanderen. [Historical ecology of forests in Flanders] (in Dutch) Davidsfonds, Leuven.

Taverna K, Peet RK, Phillips LC (2005) Long-term change in ground-layer vegetation of deciduous forests of the North Carolina Piedmont, USA. Journal of Ecology 93, 202-213.

Taylor K (1997) *Geum urbanum* L. Journal of Ecology 85, 705-720.

Taylor K (2009) Biological flora of the British isles: *Urtica dioica* L. Journal of Ecology 97, 1436-1458.

Taylor K, Woodell SRJ (2008) Biological flora of the British isles: *Primula elatior* (L.) Hill. Journal of Ecology 96, 1098-1116.

ter Braak CJF, Šmilauer P (2002) CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5). Microcomputer Power, Ithaca, USA.

Tessier JT, Raynal DJ (2003) Use of nitrogen to phosphorus ratios in plant tissue as an indicator of nutrient limitation and nitrogen saturation. Journal of Applied Ecology 40, 523-534.

Thimonier A, Dupouey JL, Bost F, Becker M (1994) Simultaneous eutrophication and acidification of a forest ecosystem in North-East France. New Phytologist 126, 533-539.

Tilman D (1982) Resource competition and community structure. Princeton University Press, Princeton, USA.

Tilman D, May RM, Lehman CL, Nowak MA (1994) Habitat destruction and the extinction debt. Nature 371, 65-66.

Turnbull LA, Crawley MJ, Rees M (2000) Are plant populations seed-limited? A review of seed sowing experiments. Oikos 88, 225-238.

Valladares F, Sanchez-Gomez D, Zavala MA (2006) Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. Journal of Ecology 94, 1103-1116.

Valladares F, Gianoli E, Gomez JM (2007) Ecological limits to plant phenotypic plasticity. New Phytologist 176, 749-763.

Valtinat K, Bruun HH, Brunet J (2008) Restoration of oak forest: Effects of former arable land use on soil chemistry and herb layer vegetation. Scandinavian Journal of Forest Research 23, 513-521.

Van Calster H (2008) Effects of changing management regimes on understorey plant communities in former coppice systems. PhD thesis, K.U.Leuven, Leuven, Belgium.

Van Calster H, Baeten L, De Schrijver A, De Keersmaeker L, Rogister JE, Verheyen K, Hermy M (2007) Management driven changes (1967-2005) in soil acidity and the understorey plant community following conversion of a coppice-with-standards forest. Forest Ecology and Management 241, 258-271.

Van Calster H, Baeten L, Verheyen K, De Keersmaeker L, Dekeyser S, Rogister JE, Hermy M (2008a) Diverging effects of overstorey conversion scenarios on the understorey vegetation in a former coppice-with-standards forest. Forest Ecology and Management 256, 519-528.

Van Calster H, Chevalier R, Van Wyngene B, Archaux F, Verheyen K, Hermy M (2008b) Long-term seed bank dynamics in a temperate forest under conversion from coppice-with-standards to high forest management. Applied Vegetation Science 11, 251-260.

van Oijen D, Feijen M, Hommel P, den Ouden J, de Waal R (2005) Effect of tree species composition on within-forest distribution of understorey species. Applied Vegetation Science 8, 155-166.

Van Ranst E, Verloo M, Demeyer A, Pauwels JM (1999) Manual for the soil chemistry and fertility laboratory. Ghent University, Ghent, Belgium.

Vellend M (2003) Habitat loss inhibits recovery of plant diversity as forests regrow. Ecology 84, 1158-1164.

Vellend M (2004) Parallel effects of land-use history on species diversity and genetic diversity of forest herbs. Ecology 85, 3043-3055.

Vellend M (2005) Land-use history and plant performance in populations of *Trillium grandiflorum*. Biological Conservation 124, 217-224.

Vellend M (2010) Conceptual synthesis in community ecology. Quarterly Review of Biology 85, 183-206.

Vellend M, Myers JA, Gardescu S, Marks PL (2003) Dispersal of *Trillium* seeds by deer: Implications for long-distance migration of forest herbs. Ecology 84, 1067-1072.

Vellend M, Verheyen K, Jacquemyn H, Kolb A, Van Calster H, Peterken G, Hermy M (2006) Extinction debt of forest plants persists for more than a century following habitat fragmentation. Ecology 87, 542-548.

Vellend M, Verheyen K, Flinn KM, Jacquemyn H, et al. (2007) Homogenization of forest plant communities and weakening of species-environment relationships via agricultural land use. Journal of Ecology 95, 565-573.

Verburg R, Grava D (1998) Differences in allocation patterns in clonal and sexual offspring in a woodland pseudo-annual. Oecologia 115, 472-477.

Verheyen K (2002) The relative importance of seed and recruitment limitation of vascular plants in secondary forest succession. PhD thesis, K.U.Leuven, Leuven, Belgium.

Verheyen K, Hermy M (2001a) An integrated analysis of the spatio-temporal colonization patterns of forest plant species. Journal of Vegetation Science 12, 567-578.

Verheyen K, Hermy M (2001b) The relative importance of dispersal limitation of vascular plants in secondary forest succession in Muizen Forest, Belgium. Journal of Ecology 89, 829-840.

Verheyen K, Hermy M (2004) Recruitment and growth of herb-layer species with different colonizing capacities in ancient and recent forests. Journal of Vegetation Science 15, 125-134.

Verheyen K, Bossuyt B, Hermy M, Tack G (1999) The land use history (1278-1990) of a mixed hardwood forest in western Belgium and its relationship with chemical soil characteristics. Journal of Biogeography 26, 1115-1128.

Verheyen K, Adriaens D, Hermy M, Deckers S (2001) High-resolution continuous soil classification using morphological soil profile descriptions. Geoderma 101, 31-48.

Verheyen K, Bossuyt B, Honnay O, Hermy M (2003a) Herbaceous plant community structure of ancient and recent forests in two contrasting forest types. Basic and Applied Ecology 4, 537-546.

Verheyen K, Guntenspergen GR, Biesbrouck B, Hermy M (2003b) An integrated analysis of the effects of past land use on forest herb colonization at the landscape scale. Journal of Ecology 91, 731-742.

Verheyen K, Honnay O, Motzkin G, Hermy M, Foster DR (2003c) Response of forest plant species to land-use change: a life-history trait-based approach. Journal of Ecology 91, 563-577.

Vesterdal L, Rsenqvist L, Johansson M-B (2002) Effect of afforestation on carbon sequestration in soil and biomass. In: Hansen K (ed) Planning afforestation on previously managed arable land – influence on deposition, nitrate leaching, precipitation surplus, and carbon sequestration. Litterature review AFFOREST project. Forest & Landscape, Horsholm, Denmark.

Wall A, Hytönen J (2005) Soil fertility of afforested arable land compared to continuously forested sites. Plant and Soil 275, 247-260.

Ward LK (1982) The conservation of juniper – Longevity and old-age. Journal of Applied Ecology 19, 917-928.

Weber N (2000) NEWFOR - New forest for Europe: afforestation at the turn of the century. EFI Proceedings No. 35, Frieburg, Germany.

Whitney GG (1994) From coastal wilderness to fruited plain. Cambridge University Press, Cambridge, UK.

Whitney GG, Foster DR (1988) Overstorey composition and age as determinants of the understorey flora of woods of central New England. Journal of Ecology 76, 867-876.

Wiegmann SM, Waller DM (2006) Fifty years of change in northern upland forest understories: Identity and traits of "winner" and "loser" plant species. Biological Conservation 129, 109-123.

Wood T, Bormann FH, Voigt GK (1984) Phosphorus cycling in a northern hardwood forest – biological and chemical control. Science 223, 391-393.

Wulf M (1997) Plant species as indicators of ancient woodland in northwestern Germany. Journal of Vegetation Science 8, 635-642.

Wulf M (2003) Preference of plant species for woodlands with differing habitat continuities. Flora 198**,** 444-460.

Young TP, Petersen DA, Clary JJ (2005) The ecology of restoration: historical links, emerging issues and unexplored realms. Ecology Letters 8, 662-673.

Zuur AF, Ieno EN, Walker N, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. Springer, New York, USA.

CURRICULUM VITAE

Education

2003–2006	MSc in Bioscience engineering, Land & Forest Management, Ghent University, Faculty of Bioscience Engineering
2001–2003	BSc in Applied Biological Sciences, University of Antwerp, Faculty of Sciences
1995–2001	Secondary School (Mathematics-Sciences), Sint-Pietersinstituut, Turnhout

Professional experience

January 2007–present	PhD research at Ghent University, Faculty of Bioscience Engineering, Department of Forest and Water Management, Laboratory of Forestry
August 2006–December 2006	Scientific worker at Ghent University, Faculty of Bioscience Engineering, Department of Forest and Water Management on the project 'Toestand en evolutie (1985-2006) van de jeneverbes (*Juniperus communis*) in de provincie Limburg'

Scientific publications

Publications in international journals with peer review cited in the Science Citation Index (IF: impact factor in 2009)

Baeten L, Verstraeten G, De Frenne P, Vanhellemont M, Wuyts K, Hermy M, Verheyen K. Former land use affects the nitrogen and phosphorus concentrations and biomass of forest herbs. Plant Ecology, accepted with minor revisions (IF 1.567)

Verstraeten G, **Baeten L**, Verheyen K. Habitat preferences of nightjar (*Caprimulgus europaeus*) in forests on sandy soils. Bird Study, accepted with minor revisions. (IF 0.966)

Baeten L, Vanhellemont M, De Frenne P, Hermy M, Verheyen K. The phosphorus legacy of former agricultural land use can affect the production of germinable seeds in forest herbs. Ecoscience, in press. doi: 10.2980/17-4-3372 (IF 1.117)

Baeten L, Velghe D, Vanhellemont M, De Frenne P, Hermy M, Verheyen K. Early trajectories of spontaneous vegetation development after intensive agricultural land use. Restoration Ecology, in press. doi: 10.1111/j.1526-100X.2009.00627.x (IF 1.665)

De Frenne P, **Baeten L**, Graae BJ, Brunet J, Wulf M, Orczewska A, Kolb A, Jansen I, Jamoneau A, et al. Interregional variation in the floristic recovery of post-agricultural forests. Journal of Ecology, in press. (IF 4.690)

De Frenne P, Graae BJ, Kolb A, Shevtsova A, **Baeten L**, Brunet J, Chabrerie O, Cousins SA, Decocq G, Dhondt R, et al. (2010) An intraspecific application of the leaf-height-seed ecology strategy scheme on forest herbs along a latitudinal gradient. Ecography, in press. doi: 10.1111/j.1600-0587.2010.06399.x (IF 4.385)

De Keersmaeker L, Vandekerkhove K, Verstraeten A, **Baeten L**, Verschelde P, Hermy M, Verheyen K. Clear-felling effects on colonization rates of shade-tolerant forest herbs into a post-agricultural forest adjacent to ancient forest. Applied Vegetation Science, in press. doi: 10.1111/j.1654-109X.2010.01101.x (IF 1.349)

Baeten L, De Frenne P, Verheyen K, Graae BJ, Hermy M (2010) Forest herbs in the face of global change: a single-species-multiple-threats approach for *Anemone nemorosa*. Plant Ecology & Evolution 143, 19-30. (IF 0.556)

Baeten L, Hermy M, Van Daele S, Verheyen K (2010) Unexpected understorey community development after 30 years in ancient and post-agricultural forests. Journal of Ecology 98, 1447-1453. (IF 4.690)

Baeten L, Vanhellemont M, De Frenne P, Hermy M, Verheyen K (2010) Plasticity to phosphorus and light availability in four forest herbs with different colonization capacities. Oecologia 163, 1021–1032. (IF 3.129)

Vanhellemont M, Wauters L, **Baeten L**, Bijlsma R-J, De Frenne P, Hermy M, Verheyen K (2010) *Prunus serotina* unleashed: invader dominance after 70 years of forest development. Biological invasions 12, 1113-1124. (IF 3.074)

Baeten L, Bauwens B, De Schrijver A, De Keersmaeker L, Van Calster H, Vandekerkhove K, Roelandt B, Beeckman H, Verheyen K (2009) Herb layer changes (1954-2000) related to the conversion of coppice-with-standards forest and soil acidification. Applied Vegetation Science 12, 187-197. (IF 1.349)

Baeten L, Hermy M, Verheyen K (2009) Environmental limitation contributes to the differential colonization capacity of two forest herbs. Journal of Vegetation Science 20, 209-223. (IF 2.376)

Baeten L, Jacquemyn H, Van Calster H, Van Beek E, Devlaeminck R, Verheyen K, Hermy M (2009) Low recruitment across life stages partly accounts for the slow colonization of forest herbs. Journal of Ecology 97, 109-117. (IF 4.690)

Vanhellemont M, **Baeten L**, Hermy M, Verheyen K (2009) The seedling bank stabilizes the erratic early regeneration. Ecoscience 16, 452–460. (IF 1.117)

Van Calster H, **Baeten L**, Verheyen K, De Keersmaeker L, Dekeyser S, Rogister JE, Hermy M (2008) Diverging effects of overstorey conversion scenarios on the understorey vegetation in a former coppice-with-standards forest. Forest Ecology and Management 256, 519-528. (IF 1.950)

Van Calster H, **Baeten L**, De Schrijver A, De Keersmaeker L, Rogister JE, Verheyen K, Hermy M (2007) Management driven changes (1967-2005) in soil acidity and the understorey plant community following conversion of a coppice-with-standards forest. *Forest Ecology and Management* 241, 258-271. (IF 1.950)

Publications in national journals with peer review

Baeten L, Vanhellemont M, Van Calster H, Hermy M, De Schrijver A, Verheyen K (2009) Zullen bosplantenpopulaties zich ooit vestigen in jonge bossen op voormalige landbouwgronden? De Levende Natuuur 110, 215-219.

Vanhellemont M, **Baeten L**, De Keersmaeker L, Vandekerkhove K, Hermy M, Verheyen K (2009) Is Amerikaanse vogelkers overal even invasief? De Levende Natuur 110, 339-343.

Van Nevel L, Velghe D, **Baeten L**, Verheyen K (2009) Exploiteren in eigen regie, waarom nie? Bosrevue 28, 1-7.

Baeten L, Bauwens B, De Schrijver A, De Keersmaeker L, Van Calster H, Vandekerkhove K, Roelandt B, Beeckman H, Verheyen K (2008) Vegetatieveranderingen in het Meerdaalwoud (1954-2000). Hoe hebben een verandering in het beheer en toegenomen verzuring de vegetatie gewijzigd? Natuur.focus 7, 40-45.

Chapters in books

Bijlsma RJ, Kindt V, den Ouden J, **Baeten L**, Verheyen K (2010) Successie en bosdynamiek. In: den Ouden J, Muys B, Mohren F, Verheyen K. (eds) Bosecologie en bosbeheer, Acco, Leuven, Belgium, pp 195-217.

Hermy M, **Baeten L**, Roelandt B, Plue J (2009) Flora en vegetatie, ook een spiegel van het verleden? In: Baeté H, De Bie M, Hermy M, Van den Bremt P (eds) Miradal. Erfgoed in Heverleebos en Meerdaalwoud, Davidsfonds, Leuven, Belgium, pp 188-215.

Verheyen K, Adriaensens S, Crabbe S, **Baeten L** (2006) Haalt de jeneverbes het jaar 2025 in Limburg. Likona jaarboek 2006 Nr 16, Provinciaal natuurcentrum, Hasselt, Belgium, pp 26-31.

Proceedings of presentations at scientific congresses

Vanhellemont M, **Baeten L**, Hermy M, Verheyen K (2010) Patterns of *Prunus serotina* invasion in two contrasting forests. Extended abstract of oral presentation. In: Segers H, Branquart E (eds) Science Facing Aliens. Proceedings of a scientific meeting on Invasive Alien Species, Belgian Biodiversity Platform, Brussels, Belgium, pp 59-63.

Baeten L, Jacquemyn H, Van Calster H, Hermy M, Verheyen K (2008) Are forest plants able to recruit and survive after introduction in post-agricultural forests? In: Proceedings SER 2008, 6th European Conference on Ecological Restoration, Society for Ecological Restoration, Ghent, Belgium, CD-ROM paper 017.

Baeten L, Hermy M, Verheyen K (2007) Differential survival and performance of forest herbs in ancient and recent forest. Communications in Applied Biological Sciences 72, 99-102.

Van Calster H, **Baeten L**, De Schrijver A, De Keersmaeker L, Rogister JE, Verheyen K, Hermy M (2006) Differential changes (1967-2005) in soil acidity and understory community driven by forest management. Verhandlungen der Gesellschaft für Ökologie 36, 202.

Abstracts of presentations at scientific congresses

Baeten L, De Frenne P, Verheyen K, Hermy M (2010) The effect of multiple global environmental changes on the performance of forest herbs: a review with *Anemone nemorosa*. Abstract of oral presentation in: Abstracts Netherlands Annual Ecology Meeting, Lunteren, The Netherlands, p 4.

Vangansbeke P, Vanhuyse K, **Baeten L**, Hermy M, Verheyen K (2010) Dertig jaar vegetatieveranderingen in het integrale bosreservaat Lady Park Wood (UK). Abstract of oral presentation in: Abstracts symposium Starters in het Natuuronderzoek 2010, Brussels, Belgium.

Baeten L, Jacquemyn H, Van Calster H, Van Beek E, Verheyen K, Hermy M (2009) Rekrutering van bosplanten in recente bossen: een introductie experiment. Abstract of poster presentation in: Abstracts symposium Starters in het Bosonderzoek 2009, Brussels, Belgium.

Boonen W, Van den Broeck T, **Baeten L**, Verheyen K, Muys B (2009) Standplaatsafhankelijke vegetatieveranderingen en bodemverzuring in bossen op zandgrond in de Gaume. Abstract of oral presentation in: Abstracts symposium Starters in het Bosonderzoek 2009, Brussels, Belgium.

De Schrijver A, **Baeten L**, De Clerck E, Thomaes A, De Keersmaeker L, Staelens J, Wuyt K, Hansen K, Vesterdahl L, Muys B, et al. (2009) Recovery of post-agricultural forest: tree species determines soil properties and possibilities for ecosystem restoration. Abstract of oral presentation in: Abstracts 6th International Symposium on Ecosystem Behaviour BIOGEOMON 2009, Helsinki, Finland, p 498.

Vanhellemont M, **Baeten L**, Hermy M, Verheyen K (2009) The invasive *Prunus serotina* stabilizes its erratic early regeneration by building up a seedling bank. Abstract of oral presentation in: Abstracts BIOLIEF: World Conference on Biological Invasions and Ecosystem Functioning BIOLIEF, Porto, Portugal, p16.

Baeten L, Hermy M, Verheyen K (2007) Recruitment limitation of herbs colonizing recent forests: results of an introduction experiment (2000-2007). Abstract of oral presentation in: Abstracts IAVS symposium 2007, 50th Annual Symposium of The International Association for Vegetation Science, Swansea, Wales, p 29.

Scientific reports

Baeten L, Verheyen K (2010) Studie naar de bodemchemie in natuurreservaat Heiblok (Zoerselbos): relatie met de landgebruiksgeschiedenis en knelpunten voor natuurontwikkeling. Ghent University, Ghent, Belgium.

Verheyen K, De Schrijver A, Staelens J, **Baeten L**, De Frenne P, Adriaenssens S, Verstraeten G, Ampoorter E, Van Nevel L, Demey A, Wuyts K, Gruwez G (2010) Pilootstudie naar kwantificering van de relaties tussen de achteruitgang van biodiversiteit en chronische overschrijding van kritische lasten. Studie in opdracht van het Instituut voor Natuur- en Bosonderzoek (Natuur-rapportering), Ghent University, Ghent, Belgium.

Bomans E, Beliën W, **Baeten L**, Ampoorter E (2009) Opstellen van een referentie- en afwegingskader voor natuurontwikkeling en -herstel op vermeste en verzuurde terreinen. Eindrapport project P/00/050. Belgian Soil Service, Tienen, Belgium.

Adriaensens S, **Baeten L**, Crabbe S, Verheyen K (2006) Toestand en evolutie (1985–2006) van de jeneverbes (*Juniperus communis*) in de provincie Limburg. Ghent University & LIKONA, Ghent, Belgium.

MSc thesis

Baeten L (2006) Veranderingen in de kruidlaag (1967-2005) in het domeinbos Tournibus (houtvesterij Philippeville). MSc thesis, Ghent University, Ghent, Belgium.

Scientific activities

Participation in congresses, symposia or workshops

Participation with oral presentation

2-4 November 2010. Design exploratory platform FunDivEUROPE. Functional significance of forest biodiversity in Europe, Kick-off meeting (Freiburg University), Freiburg, Germany.

27 May 2010. De verspreiding van de flora in het Muizenbos: een unieke case van kolonisatie door bosplanten. Voorstelling van het onderzoek in bosreservaat Muizenbos (INBO), Ranst, Belgium.

9-10 February 2010. The effect of multiple global environmental changes on the performance of forest herbs: a review with *Anemone nemorosa*. The Netherlands Annual Ecology Meeting (NERN, Necov), Lunteren, the Netherlands.

2 October 2009. Long-term vegetation changes in northwestern European forests. Woodland monitoring workshop (British Ecological Society), Cambridge University, Cambridge, UK.

27 January 2009. Ecologische aspecten van duurzaam bosbeheer: lessen uit de kruidlaag. Symposium Natuurwaarde in bos: wat is de evolutie en hoe wordt die beïnvloed door het gevoerde beheer (Pro Silva, Bossenwerkgroep), Antwerp, Belgium.

8-12 September 2008. Are forest plants able to recruit and survive after introduction in post-agricultural forests? 6th European Conference on Ecological Restoration. Towards a sustainable future for European ecosystems – Providing restoration guidelines for Natura 2000 habitats and species. (Society for Ecological Restoration, INBO), Ghent, Belgium.

23-27 July 2007. Recruitment limitation of forest herbs colonizing recent forests: long-term (2000–2007) results of an introduction experiment. 50th annual symposium of the international association for Vegetation Science. Cultural landscapes – changing landscapes (IAVS), Swansea University, Swansea, UK.

22 March 2007. Veranderingen in de kruidlaag (1967-2005) van het domeinbos Tournibus door een gewijzigd bosbeheer. Starters in het bosonderzoek 2007 (Afd. Bos- en Groen, INVERDE), Brussels, Belgium.

Participation with poster presentation

19 March 2009. Rekrutering van bosplanten in recente bossen: een introductie experiment. Starters in het bosonderzoek 2009 (ANB, INVERDE), Brussels, Belgium.

30 May 2008. Low recruitment success contributes to the slow recovery of forest plant populations in post-agricultural forest. Symposium Aardse Zaken (Dpt. Earth & Environmental Sciences K.U.Leuven), Leuven, Belgium.

17 October 2007. Differential survival and performance of forest herbs in ancient and recent forest. 13th PhD Symposium on Applied Biological Sciences (Faculties Applied Biological Sciences K.U.Leuven and Ghent University), Leuven, Belgium.

Participation without presentation

24 November 2009. Workshop "Tree diversity experiments: status, results, joint analyses" (Tree Diversity Network), Ghent University, Ghent, Belgium.

14-15 September 2009. 4th international symposium on dispersal. Organisms on the move (The Internations Dispersal Workgroup), Ghent, Belgium.

8 February 2008. NECOV – wintersymposion 2008. Timeless Ecology: from seconds to centuries (Necov), Antwerp, Belgium.

Supervision MSc thesis students

2009–2010 Pieter Vangansbeke (UGent). Thirty years of change (1979–2009) in the tree layer of the forest reserve Lady Park Wood (UK). Supervisors: Prof. Dr. Ir. Kris Verheyen, Prof. Dr. Martin Hermy.

Kathleen Vanhuyse (K.U.Leuven). Changes (1979–2009) in the herbaceous layer of the strict forest reserve Lady Park Wood (UK). Supervisors: Prof. Dr. Martin Hermy, Prof. Dr. Ir. Kris Verheyen.

2008–2009 Wim Boonen (K.U.Leuven). Bodemverzuring in bossen van mesotrofe naar oligotrofe staat: op zoek naar indicatoren van regimeverschuiving. Supervisors: Prof. Dr. Ir. Bart Muys, Prof. Dr. Ir. Kris Verheyen.

Sander Van Daele (UGent) 30 jaar vegetatiedynamiek in jonge en oude bossen in het westelijk deel van Vlaanderen. Supervisors: Prof. Dr. Ir. Kris Verheyen, Prof. Dr. Martin Hermy.

Tine Van den Broeck (UGent). Vijf decennia vegetatieveranderingen in bossen op zandgrond in de Gaume (prov. Luxemburg). Supervisors: Prof. Dr. Ir. Kris Verheyen, Prof. Dr. Ir. Bart Muys.

Supervision of BSc thesis students

2009–2010 Sebastiaan Hanouille (Katholieke Hogeschool Roeselare). Analyse van bodem en vegetatie in de Heiblok (Zoerselbos). Supervisors: Dr. Ir. Dieter Anseeuw, Prof. Dr. Ir. Kris Verheyen.

2008–2009 Sanne De Smet (Universiteit Wageningen). Internship plant and systems ecology. Case 2: changes in the vegetation of a deciduous forest (1982–2008). Supervisors: Dr. Ir. Jan Bokdam, Prof. Dr. Ir. Kris Verheyen.

2007–2008 Gijs Bracke, Ellemie Comeyne, Thomas Timmerman, Michiel Van Gestel (UGent). Bebossing van Landbouwgrond: actuele situatie en potenties voor natuurherstel. Supervisor: Prof. Dr. Ir. Kris Verheyen.

Review tasks for international journals

2010	Evolutionary Ecology (1), Plant Ecology (1), Forest Ecology and Management (1)
2009	Diversity & Distributions (1), Journal of Ecology (1)
2008	Applied Vegetation Science (1), Forest Ecology and Management (1), Plant Ecology (1), Scandinavian Journal of Forest Research (1)
2007	Acta Oecologica (1), Journal of Environmental Management (1)

ISBN 978-90-5989-405-1