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Tree species effects on herb layer development in post-agricultural forests

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Dutch translation of the title:
Boomsoorteneffect op de ontwikkeling van de kruidlaag op beboste landbouwgronden

Illustrations on the cover:
Front: *Primula elatior* introduced in the Mortagne forest next to *Urtica dioica*
Back: *Anemone nemorosa* introduced in the Mortagne forest

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Woord vooraf

Nothing ever grows without a seed – Debby Boone

Een lange weg komt tot een einde. Van jongs af ben ik gefascineerd door het zaaien van planten. Als master thesis wilde ik dan ook oudbosplanten zaaien. Zo kwam ik in 1999 bij Luc terecht, een leerrijke en inspirerende periode. Onvergetelijk hoe we samen in zijn auto enkele honderden kilo grond uit het Mortagnebos gingen halen. Na mijn afstuderen, kon ik aan het IBW/INBO beginnen. Hier kon ik vanaf 2004 de draad opnieuw oppikken nadat Bos & Groen mijn project rond landbouwbebossingen goedkeurde. Later kwamen er andere opdrachten bij zodat de eindstreep soms heel ver leek.

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Ondertussen staan er nieuwe uitdagingen voor de deur al is het op dit moment nog niet geheel duidelijk welk strategisch of andere onderzoek ik morgen zal opstarten. In Sint-Niklaas wacht een zadenbib op zijn opstart, dus mijn interesse in zaden krijgt alvast een nieuwe uitlaatklep. Debby, zet die verfpotten maar al klaar, ik ga de gang schilderen, zeker nog dit jaar.

Men love to wonder, and that is the seed of science – Ralph Waldo Emerson

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Summary

The forest cover in Flanders and many similar lowland regions has been subjected to many land use changes in previous centuries. Especially on fertile loam and sand loam soils, the forest cover has been declining rather continuously. Only in the last decades, fertile agricultural land has been afforested for biodiversity conservation and other objectives.

These so called post-agricultural forests have, however, strongly altered soil characteristics, especially elevated phosphorus (P) levels. The elevated P concentrations cause a strong increase in growth of competitive species, like *Urtica dioica* L.. While, stress tolerant species like many forest plant species, show only a limited response in growth.

Many forest plant species, so called ancient forest species (AFS), are predominantly present in sites with a long and continuous forest land use due to their limited colonisation capacity of recent forest. It concerns a large group of species and consequently they are important for the functional and taxonomic biodiversity of the herb layer of forests. The colonisation of AFS in post-agricultural forests can thus be considered as a bottleneck for forest restoration.

In this thesis, tree species effects on the colonisation of AFS are studied in post-agricultural forests. Tree species are known to have a strong impact on soil characteristics, light transmittance and understory vegetation. It is hypothesized that increased shade levels will suppress competitors and facilitate the colonisation of AFS while acidifying tree species will strongly limit the survival of many AFS.

The thesis comprises an observational vegetation study and four different experiments focusing on tree species effects on AFS. The vegetation study showed a diverging vegetation development in a chronosequence with two contrasting tree species, i.e. poplar and oak. This is explained by the quick soil acidification under oak compared to stable near neutral pH under poplar. No difference was found in the cover of forest plants, but oak had mainly acid tolerant forest plants while poplars had more acid intolerant forest plants. For the first experiment six AFS were introduced under eight different tree species in a common garden. The survival of the introduced AFS was limited by soil acidifying tree species while growth of these AFS was higher under tree species with low light levels. Temporary canopy gaps promoted the establishment of AFS. In a sowing experiment, germination and recruitment of both acid tolerant and intolerant species was higher under tree species with higher soil pH. However, competitive species from the seed bank also germinated more numerously by higher pH. In another sowing experiment, recruitment showed to be independent for early and late leafing out shrub species. However, four out of seven species showed higher recruitment in shaded versus non shaded treatment. In a final experiment, tree species induced soil acidification is linked to decreasing plant Ca and P concentrations and increasing Mg and Al shoot/root ratios, likely explained by Al antagonism. A potential interaction between light availability and soil acidification effects was not found in the various experiments.

The results lead to the conclusion that tree species are indeed important drivers of the understory development in post-agricultural forests. This tree species effect can be explained by their impact on soil acidification and understory light availability. Dark stands suppress the competitors and facilitate the vegetative colonisation of many AFS. Temporary canopy gaps seem to facilitate recruitment of AFS. However, it is important that gaps close quickly enough to prevent competitors from establishing. The recruitment and survival of acid intolerant AFS is strongly limited to moderately acidifying tree species.

In the concluding chapter, a simple framework is proposed to evaluate tree species effects based on light transmittance and soil acidity. It is concluded that topsoil is best kept clearly above pH-H_2O 4.2 and light transmittance below 8% of the open field for the optimal recovery of AFS.

Samenvatting

Het bosareaal in Vlaanderen en vele gelijkaardige laaglanden heeft sterke verschuivingen gekend in landgebruik in de vorige eeuwen. Vooral op vruchtbare leem en zandleemgronden, heeft het bosareaal een redelijk onafgebroken daling gekend. Pas in de laatste decennia, wordt landbouwgrond bebost voor biodiversiteitsbehoud en andere doelstellingen.

Deze beboste landbouwgronden hebben echter sterk veranderde bodemkenmerken, in het bijzonder sterk verhoogde fosfor (P) concentraties. De verhoogde P concentraties veroorzaken een sterke toename van de groei van competitieve soorten, zoals Grote brandnetel. Terwijl, stresstolerante soorten zoals vele bosplanten slechts beperkte toename in groei vertonen.

Veel bosplanten, zogenaamde oudbosplanten, zijn voornamelijk te vinden in gebieden met een lang en continu landgebruik als bos, dit door hun beperkte kolonisatiecapaciteit van recente bebossingen. Het betreft een groot aantal soorten en bijgevolg zijn ze belangrijk voor de functionele en taxonomische biodiversiteit in de kruidlaag van bossen. De kolonisatie van oudbosplanten in landbouwbebossingen kan dus beschouwd worden als een knelpunt voor het herstel van boshabitats.

In deze thesis wordt het boomsoorteneffect op de kolonisatie van oudbosplanten bestudeerd in landbouwbebossingen. Het is bekend dat boomsoorten een sterke invloed hebben op de kenmerken van de bodem, lichtdoorval en oeverbegroeiing. Als hypothese wordt gesteld dat een diepere schaduw competitieve soorten kan onderdrukken en de kolonisatie van oudbosplanten kan vergemakkelijken, terwijl verzurende boomsoorten de vestiging en overleven van vele oudbosplanten sterk zal beperken.

De thesis omvat een observationele studie van de vegetatie en vier verschillende experimenten over het boomsoorteneffect op oudbosplanten. De vegetatie studie toont een divergerende vegetatie-ontwikkeling aan in een chronosequentie van twee contrasterende

boomsoorten, namelijk populier en eik. Dit wordt verklaard door de snelle bodemverzuring onder eik in vergelijking met de bijna neutrale pH onder populier. Er werden geen verschillen gevonden in de bedekking met bosplanten, onder eik stonden voornamelijk zuur tolerante bosplanten terwijl onder populier meer zuur intolerante bosplanten stonden. In het eerste experiment werden zes oudbosplanten geïntroduceerd in een proefaanplant met acht verschillende boomsoorten. De overleving van de geïntroduceerde planten werd beperkt bij de bodem verzurende boomsoorten. Terwijl de groei van deze oudbosplanten hoger was onder boomsoorten met lage lichtbeschikbaarheid. Tijdelijke gaten in het kronendak bevorderden de vestiging van oudbosplanten. In een zaai experiment was de kieming en vestiging van zowel zuur tolerante als intolerante oudbosplanten hoger onder boomsoorten met een hogere bodem pH. Maar ook de competitieve soorten kiemden meer talrijk uit de zaadbank bij hogere bodem pH. Een ander zaai experiment toonde aan dat de vestiging onafhankelijk is van vroeg en laat uitlopende struiksoorten. Vier van de zeven soorten vertoonden echter wel een hogere vestiging in beschaduwde dan onbeschaduwde proefvlakken. In een laatste experiment werd de bodemverzuring veroorzaakt door verschillende bomsoorten, gelinkt met dalende plantenconcentraties van calcium en fosfor en stijgende magnesium en aluminium scheut/wortel concentratieverhoudingen, wellicht verklaard door aluminium antagonisme. Een potentiele interactie tussen licht beschikbaarheid en bodemverzuring werd niet gevonden in de verschillende experimenten.

De resultaten leiden tot de conclusie dat boomsoorten inderdaad belangrijke stuurvariabelen zijn voor de kruidlaagontwikkeling in beboste landbouwgronden. Dit boomsoorteneffect kan worden verklaard door het effect op de bodem verzuring en beschikbaarheid licht. Donkere bestanden onderdrukken de competitoren en vergemakkelijken de kolonisatie van vele oudbosplanten. Tijdelijke gaten in het kronendak lijken de vestiging van oudbosplanten niettemin te stimuleren. Het is echter belangrijk dat deze gaten snel genoeg dichtgroeien om de vestiging van competitoren te verhinderen. Zuurintolerante oudbosplanten zijn sterk beperkt tot matig verzurende boomsoorten.

In de conclusies wordt een eenvoudig schema voorgesteld om boomsoorteneffect te evalueren op basis van de lichtdoorval en bodem zuurtegraad. Er wordt geconcludeerd dat de zuurtegraad van de bovenste bodemlaag best duidelijk boven pH-H_2O 4.2 wordt gehouden en de lichtdoorval onder de 8% van het vrije veld voor het optimaal herstel van oudbosplanten.

List of abbreviations

∑herblayer: Sum of the cover of the herb layer species
*: $p < 0.05$
**: $p < 0.01$
***: $p < 0.001$
%Overstory: Cover of the shrub and tree layer
%S: Sand fraction
%Understory: Cover of the herb layer
ACID: Acidifying tree species
acidFS: Acid tolerant forest herb species ($R< 6.5$)
acidintolFS: Acid intolerant forest herb species ($R\geq 6.5$)
Adj: Adjacent to ancient forest
AFS: Ancient forest plant species
Age: Stand age
Al: Aluminium
ANOVA: Analysis of variance
bio-P: Soil bio-available phosphorus concentration
C: Carbon
C-herb: C fraction of Hunt CSR plant strategy from the herb layer
Ca: Calcium
cf.: Conform
Ch: Chamaephyte
Chi^2: Chi-squared test
CLEAR: Clearcut
Cr: Crop field
CSR: Signature of the vegetation based on the competitiveness, stress-tolerance and ruderality strategy
df: Degrees of freedom
e.g.: Exempli gratia: Example given
EDTA: Ethylenediaminetetraacetic acid
et al.: Et alii: And others
F: F-test
Fe: Iron
FL: Former land use
FS: Forest species (list from Honnay et al. 1998 annex 1)
GAM: Generalised additive model(s)
Ge: Geophyte
GERM: Germination
GLMM: Generalised linear mixed model(s)
Gr: Grassland
H: Hemicryptophyte
H^+: Proton
ha: Hectare
i.e.: Id est: That is
Iso: Isolated from ancient forest
k: K-value of a smoother in GAM
K: Potassium
Kj-N: Kjeldahl nitrogen concentration
LAI: Leaf area index
lightFS: Light demanding forest edge species (list from Honnay et al. 1998 annex 1)
Lll: Length of longest leaf
Llp: Length of longest pedicel
LMM: Linear mixed model(s)
Mg: Magnesium
mL: Mean Ellenberg value for light
mN: Mean Ellenberg value for nitrogen
MOAC: Moderately acidifying tree species
MOSH: Moderately shaded
mR: Mean Ellenberg value for reaction (soil pH)
n: Sample size
N: Nitrogen
N_2: Nitrogen gas
NA: Not applied
Nf: Number of flowers
Nl: Number of leaves
Np: Number of pedicels
NS: Not significant

Nseedl: Number of seedlings
p: Probability-value
P: Phosphorus
PCA: Principal component analysis
PERMANOVA: Permutational multivariate analysis of variance
pH: Power of hydrogen: measurement of the proton activity
pH-deep: pH-H_2O at a soil depth of 45-55 cm
PlC: Plant cover
PlH: Plant height
R: Ellenberg value for acidity
R: R statistic program
S-herb: S fraction of Hunt CSR plant strategy from the herb layer
s.d.: Standard deviation
s.d.: Sine die: No date
s.e.: Standard error
shadeFS: Shade tolerant forest species (list from Honnay et al. 1998 annex 1)
STSH: Strongly shaded
SURV: Survival
t: T-test
tF: Time period of first seedlings
tH: Time period of highest number of seedlings
tot-P: Total soil phosphorus concentration
TS: Tree species
VIF: Variance inflation factor
vs.: Versus
yr: Year

Flora: Species names follow Van Landuyt et al. (2012)

1 Introduction

1.1 Forest cover reduction and expansion

1.1.1 Forest cover reduction

Without human interference most of Europe would be covered with forests. Today, the forest cover in Europe is 45% but this strongly differs regionally (FAO 2010). Especially the northwestern European lowlands are remarkably poor in forest. The Netherlands (forest cover of 11%), Ireland (11%), the UK (12%), Denmark (13%) and Belgium (22%) are all among the countries with the lowest forest cover in Europe (FAO 2010). Within Belgium there is a strong regional differentiation, with a forest cover of 33% in Wallonia (Laurent et al. 2007) and 10.8% in Flanders (Bos & Groen 2000). Even larger differences exist at smaller scales, e.g. 2.3% forest cover in the province of West-Flanders to 20.6% in the province of Limburg. The low forest cover of the northwestern European lowlands is explained by the long human occupation and the high population densities (Rackham 1980; Tack et al. 1993).

Especially the area of ancient forest (i.e. forest that has not been cleared for other land-uses since a specified threshold date, often the date of the oldest available topographical maps, Peterken 1977) has reduced dramatically during the last centuries. This is most pronounced on sites which are very suitable for agriculture, e.g. fertile luvisols. Consequently rich mesophilous forest types typically growing on rich soils are most threatened (Foster 1992; Kirby and Watkins 1998; De Keersmaeker et al. 2004; Wulf et al. 2010). As a result of frequent land use shifts, only 16% of the current forest in Flanders is ancient (De Keersmaeker 2013). Furthermore, the decline in forest cover resulted in strong fragmentation of the forest cover in Flanders (e.g. De Keersmaeker 2013).

1.1.2 Forest cover expansion

While the forest cover has often nearly continuously declined during the last centuries on rich soils, many sites that were of marginal use for agriculture have been reforested. Conifer forests were planted on heathlands and poplar forest on meadows mainly in the 19th and 20th century (Foster 1992; Skalos et al. 2012; De Keersmaeker 2013). In some other regions forest expansion was the result of natural reforestation after land abandonment rather than active conversion to forest (e.g. Vojta and Drhovská 2012; Müller et al. 2013).

More recently, new objectives for afforestation have been arising, like biodiversity conservation, recreation, water purification, visual and noise buffer, carbon sequestration and many other ecosystem services (FAO 2012). These new objectives have led to altered methods and locations for afforestation compared to the afforestation of marginal lands in the past. The locations of these new forests are mainly prioritised based on, for instance, distances to cities for recreational forests (e.g. Benson and Willis 1993; Moons et al. 2008) and remnants of ancient forests for nature conservation (e.g. Brunet 2007; De keersmaeker 2013). As a consequence many of these forests are planted upon well fertilised and valuable soils from an agricultural perspective.

At the European level, financial stimuli to turn farmland back into forest as set aside policy of agricultural land have been established since 1990. Three subsequent programs of the European Agricultural Fund for Rural Development have included afforestation grants. As a result, 5.000 km² have been afforested within the European Union between 1993 and 1997 and 1.000 km² between 2000 and 2006. The third program (1698/2005) runs from 2007 to 2013. While the goal of the first program was still clearly converting marginal agricultural land to more productive forests, the latest program focuses on the protection of the environment, the prevention of natural hazards and fires, as well as on mitigating climate change (Zanchi et al. 2007).

At the national level, Ireland and Denmark can be mentioned as good examples for forest expansion. Ireland set a target in 1948 of increasing the forest area with 400.000 ha over 40 year (yr). At that time the forest cover was less than 1%. Between 1997 and 2007 11.560 ha were afforested per year in Ireland (Malone 2008). Another example is Denmark, where the goal was set in 1989 to double the forest area over a century. Therefore, an annual afforestation of 5000 ha of farmland was needed (Helles and Linddal 1996). The three main targets were groundwater protection, outdoor recreation (urban forest) and creating an ecological network. Between 1991 and 2000 about 27.500 ha were afforested (Madsen 2002; Salazar et al. 2013). Also in the Netherlands (Dirkse et al. 2006), the UK (Forestry Commission 2012), South Sweden (Helles and Linddal 1996) and in many other countries and regions large areas of fertile agricultural land have been reforested over the last decades.

In Flanders a goal of 10.000 ha of newly created forests was set in 1994 in the ‘Ruimtelijk Structuurplan Vlaanderen’. Furthermore, all deforestations needed compensation by new afforestations. The focus of this afforestation project is both on recreation and nature conservation. In spite of not reaching the objectives, about 4.000 ha of mainly agricultural land was afforested between 1994 and 2012 (BOS+ 2012).

Also the Flemish Natura 2000 goals include the creation of 6.000 to 9.000 ha of forest habitat trough afforestation as buffers and corridors for existing forest habitat, mostly ancient forest remnants (Thomaes et al. 2009). To realise this, it will be necessary to restore forest habitat, including their typical species, on former agricultural land.

Furthermore, forest species in ancient mesophilous forests are often threatened by acidification through atmospheric deposition and the promotion of tree species which provoke acid soils (Thimonier et al. 1994; Falkengren-Grerup and Diekmann 2003; Van Calster et al. 2007; Baeten et al. 2009a). Some authors have suggested that forests expansion on agricultural land can contribute to form refuges for acidification sensitive forest species (Dzwonko and Loster 1997; Bossuyt et al. 1999b; Brunet et al. 2000; van Oijen et al. 2005; Valtinat et al. 2008).

1.1.3 Post-agricultural forests

Peterken (1977) made a clear distinction between ancient and recent forest on the one hand and primary and secondary forest on the other hand as different terms and definitions were used interchangeably at that time. Peterken (1977) defined primary forests as continuously forested since before the original post-glacial forests in the region were fragmented. In contrast, secondary forests have been cleared at some time in the past to give it a different land use, e.g. arable, pasture or meadow. The evidence of the origin of older secondary forests may be difficult to obtain. This makes it difficult to claim a forest to be primary, especially in regions with a long intensive human intervention as in the northwestern European lowlands. In these situations the term ancient forest is easier to apply as it is defined as a forest that at least predates a certain threshold date. In contrast to ancient forest, the term recent forest is used, defined as a forest that is younger than the threshold date. Often, the date of the oldest topographical maps is used as threshold date (Peterken 1996, Hermy & Verheyen 2007). Recent forests are a subset of secondary forests whereas ancient forest can be either secondary or primary forest (Peterken 1996).

Since the last two decades, an increasing number of studies are using the term ‘post-agricultural forests’. This term was used for the first time by Smith et al. (1993), according to Web of Knowledge. They described the land-use history of Tompkins County (NY, USA) and used the term to distinguish between primary forest (which they defined as forest that originates from before the first settlements) and land that has been cleared for agricultural use and is afforested afterwards. In this paper and many other American studies, post-agricultural forest may be seen as a synonym of secondary forests, only emphasizing the agricultural use before the afforestation. Also Vellend (2003) defines post-agricultural forest as opposite to primary forest. The more recent use of the term in European publications differs somewhat from the American publications. Baeten (2010) defines it as forests established on former agricultural land since the oldest available historical data and highlights their enriched nutrient status.

Within this thesis, only a subset of post-agricultural forests will be studied which are only a few decades old and on formerly ploughed, well fertilized and limed soils (cf. Baeten 2010). The nutrient status of these afforestations contrasts with afforestations on poorly fertilised pastures, meadows and heathland (Kopecky & Vojta 2009, Dyer 2010).

1.1.4 Soils of post-agricultural forests

Post-agricultural forest soils are mostly differentiated from ancient forest soils by elevated soil pH and P concentrations and lower nitrogen (N) and carbon (C) concentrations (De Schrijver et al. 2012a; Hooker and Compton 2003; Koerner et al. 1997; Ritter et al. 2003; Vesterdal et al. 2002; Zhang et al. 2010). The elevated pH is the result of the liming of the former farmland and decreases again after conversion to forest.

Since the second half of the 20th century, agricultural practice has strongly intensified in northwestern Europe. Especially the increased amounts of chemical and organic fertilisation after the Second World War have led to an increased soil eutrophication. Consequently, surplus Phosphorus (P), which is not taken up by plants, has been accumulating over many years in agricultural land (Sattari et al. 2012). As a consequence high amounts of soil P (Box 1) are typically found in these more recent afforestations (Koerner et al. 1997; Verheyen et al. 1999; De Keersmaeker et al. 2004; Falkengren-Grerup et al. 2006; MacDonald et al. 2012). Hence, the soil in these post-agricultural forests does not only differ from ancient forests, they also differ from older recent forests that predate the Second World War. Elevated P concentrations, which are particularly persistent, can remain remarkably elevated for hundreds or thousands of years (Verheyen et al. 1999; Dupouey et al. 2002; Dambrine et al. 2007).

After afforestation, soil organic matter starts to increase by litter decomposition, root turnover and increased microbial activity. This results in a shift from inorganic to organic forms of P (De Schrijver et al. 2012b). The organic P fraction increases from less than 5% to 30-40% after 35 yr. Meanwhile, the labile P fraction decreases from more than 20% to less than 10% in *Quercus robur* L. afforestation. Only in the 0-5 cm of the topsoil, which is most relevant for the herb layer, the labile P fraction remains persistently high. This is explained by a stronger increase of the labile organic fraction compared to deeper soil depths. Storage of P in the woody biomass amounts only about 40 kg and is thus negligible compared to the soil P content (De Schrijver et al. 2012b).

Box 1: Soil phosphorus forms

P can be present in various forms in the soil: occluded or fixed (e.g. Fluor apatite), slowly cycling (e.g. various Calcium- (Ca), Iron- (Fe) and Al-phosphates) and labile to soluble sources (e.g. $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-}). The labile and soluble fractions are further referred to as bio-available P (bio-P). Furthermore, soil P can be found in organic and inorganic form. Soluble P is found as an anion and is adsorbed to the negatively loaded clay-humus complex. Hence, only after severe accumulation, limited amounts of P leach to the subsoil or groundwater. Only a small proportion of the total P pool is in soluble form and thus immediately available for plants and micro-organisms. When soluble P is taken up by plants, P is quickly transferred from the labile pool (and to a lesser extent from other pools) to restore the equilibrium between pools. Vice versa, when soluble P is added, large parts of it are immobilized in other P forms. In neutral and alkaline soils, Ca-phosphates tend to accumulate, whereas Fe- and Al-phosphates are predominant in acid soils. As a consequence P retention depends on the soil acidity. The optimum level for P availability is found at pH-H_2O 6.5, while there are two minima for P availability at pH-H_2O 5-5.5 and around 7.5 (Stevenson and Cole 1999).

N in soils is mainly present in organic form. Through mineralisation of the organic matter, N is set free as NH_4^+ which is subsequently transformed to NO_3^- by nitrification. Mineral forms of nitrogen that are not taken up by plants or other biota will leach out or in anaerobic conditions denitrify to N_2 gas. Consequently, N will not build up in agricultural soils as P does. After afforestation, the N input by mineralisation and deposition is initially largely fixed in the growing biomass (trees, litter layer and soil organic matter) and consequently the N leaching becomes negligible (Hansen et al. 2007). With the increasing amount of organic matter in the soil, both C and N concentration increase with the age of the afforestations, whereas the soil C/N ratio remains rather constant (Hooker and Compton 2003; Ritter et al. 2003; Zhang et al. 2010; Li et al. 2012). After about 20y, the amount of N leaching increases again due to increased deposition with increasing canopy development and decreased N demand once the most N-rich biomass compartments have been built up (Hansen et al. 2007). In most of the forests in Flanders N deposition still exceeds the critical loads for the biodiversity but currently the yearly deposition is decreasing (Overloop et al. 2011).

1.2 Forest restoration and ancient forest species

1.2.1 Forest restoration

Ecological restoration, including forest restoration, is defined as the ‘process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed’ (SER 2004). Nine attributes have been listed as an obligatory basis of what needs to be included in restoration. The first (1) attribute is ‘The restored ecosystem contains a characteristic assemblage of the species that occur in the reference ecosystem and that provide appropriate community structure.’ The eight other attributes focus on (2) the nativeness of species, (3) presence of functional groups, the restoration of the (4) physical environment and (5) ecological functioning, (6) the integration in the landscape, (7) the elimination of threats, (8) the resilience and (9) self-sustaining (SER 2004). This thesis focus on the restoration of characteristic species (first attribute), but it leaves no doubt that the realisation of this objective both depends on and determines the realisations of most of the other objectives.

Ecological restoration is an increasingly important strategy for nature conservation (Dobson et al. 1997; Young 2000). Restoration is mainly used for stabilising degraded soils, safeguarding biodiversity and habitats, reducing poverty and carbon sequestration (McBride et al. 2010 and references therein). Reforestation is one of the most used restoration techniques (Aerts and Honnay 2011; Holl and Aide 2011).

1.2.2 Ancient forest species

Ancient forest species (AFS) are identified as forest species that slowly colonise new forest habitats and are predominantly present in sites with a long and continuous forest land use (Hermy et al. 1999). Principally, this could include both fauna and flora. However, little research has focused on fauna (e.g. carabid beetles: Desender et al. 1999; slugs: Kappes 2006; weevils: Buse 2012). In the rest of this thesis, I will limit AFS to vascular plant species. Based on a review of 22 studies, Hermy et al. (1999) concluded that one third of all species found in the interior of European temperate deciduous forests are AFS. This study clarified that AFS tend to be more shade tolerant than other forest species. Furthermore, they avoid both dry and wet sites (also in De Keersmaeker et al. 2013) and most species prefer weakly acid to neutral soils and intermediate soil nutrient levels. More than a third of the AFS are primary stress tolerant species (sensu Grime et al. 1988), while only 11% do not include stress adaptation in their plant strategy (e.g. competitors, Hermy et al. 1999).

Most AFS can be seen as forest plants that are strongly adapted to a stable habitat with deep shade which they find in the forest interior (Hermy et al 1999; Verheyen et al. 2003b). Many species show either shade avoidance, by adjusting their phenology to match the higher light availability in the early spring, or shade tolerance by developing leaves with a low light compensation point (Rothstein and Zak 2001). Large seeds are necessary to recruit in shady environments with thick litter layers which limit the number of seeds that are produced, the dispersal of the seeds and their longevity (Leishman and Westoby 1994; Eriksson 1995; Verheyen et al. 2003b; Jankowska-Blaszczuk and Daws 2007). In large undisturbed forest, the limited dispersal and seed longevity will give these plants little disadvantages (Brown and Oosterhuis 1981; Hermy 1994; Grashof-Bokdam 1997).

As stress tolerant species, AFS react little to gradients of stress or nutrient levels in comparison with competitors (Grime et al. 1988). A nice example is found in the experiment of Pigott and Taylor (1964), there *Mercurialis perennis* L., an AFS, showed only a small growth increase by P supply in contrast to the competitor, *Urtica dioica* L., which showed a strong increased growth. Similarly, AFS also show a limited reaction in growth to increased light levels (Thomas et al. 1999; Lindh 2008). However, flowering of AFS increases rapidly in response to increased light levels (Lindh 2008; Baeten et al. 2010). AFS do not prefer deep shade but tolerate it and are often restricted to shade because they depend on the absence of disturbances (e.g. grazing, soil disruption) and competitors (Rackham 1980; Brunet 1993).

As a result of the low seed (or propagule) production, limited seed dispersal and longevity and the limited response to increased light and nutrients, AFS are strongly limited to establish new populations in post-agricultural forests (Ehrlén and Eriksson 2000; Verheyen et al. 2003a; Hermy and Verheyen 2007). As a broad group of species they are important for the taxonomic as well as the functional biodiversity of forests (Gilliam 2007). As characteristic and diagnostic species of various habitat types (e.g. Hermy et al. 1999; De Keersmaeker et al. 2013), they should be included in the ecological restoration of forest ecosystems. Therefore, the slow colonisation of AFS can be considered as limiting the success of forest restoration (Honnay et al. 2002a).

The group of AFS should not be seen as a fixed list of species that strongly differ from other forest species. A gradient is found in the colonising capacity of forest species which can be attributed to differences in dispersal and environmental limitation (Verheyen and Hermy 2001b; Verheyen et al. 2003b; De Frenne et al. 2011). Many earlier literature (e.g. Ehrlén and Eriksson 2000; Verheyen et al. 2003a; Graae et al. 2004; Hermy and Verheyen 2007) referred to recruitment limitation instead of environmental limitation but intrinsically mean the same. Furthermore, regional differences can be found for certain species in their dependence for ancient forest (Hermy et al. 1999; De Frenne et al. 2011). Consequently, Hermy et al. (1999) concluded that lists of AFS should come from regional studies. De Keersmaeker et al. (2011a) published a list of 44 AFS for Flanders based on nearly 4200 forest plots in ancient and recent forest compiled for a study on forest typology (Cornelis et al. 2009).

1.2.3 Impact of phosphorus enrichment on forest restoration

As mentioned earlier, post-agricultural forest are characterised by high amounts of accumulated P. This P enrichment in particular has been found as a key factor limiting biodiversity restoration (e.g. Noe et al. 2001; Wassen et al. 2005). Due to P enrichment, competition between target species of restoration and competitors is outbalanced (Menges 2008). For example, performance of stress tolerants and habitat specialists in restored grassland were affected by residual P fertility (Pywell et al. 2003; Smith et al. 2003). The high P concentration is also recognised to limit the development of typical forest plant communities (Koerner et al. 1997; Bossuyt et al. 1999b; Honnay et al. 1999a; Verheyen and Hermy 2004). Baeten et al. (2010) found that excess P on its own did not hamper AFS. In contrast, plant P concentrations increased plant performances and P uptake of some species. However, high P availability promotes P-opportunistic competitors such as *U. dioica* much more (Pigott 1971) which can suppress AFS through competition (Koerner et al. 1997; Honnay et al. 1999b; Verheyen and Hermy 2001a; De Keersmaeker et al. 2004; Baeten et al. 2009b; Orczewska 2009).

1.3 Tree species choice in forest restoration projects

Since AFS encompass a large part of the floristic biodiversity of forest habitats, the colonisation of these species is essential for successful ecological restoration of forests. Ecological restoration should, therefore, be able to solve both the propagule dispersal barriers as well as the habitat unsuitability of post-agricultural forests for AFS. Methods solving the dispersal limitation can be realized by spreading habitat restoration efforts in time and space gradually outwards from the propagule sources (e.g. Hermy et al. 1999;

Herault et al. 2005). This spatial allocation of potential sites for the colonisation of AFS has recently been applied for Flanders (De Keersmaeker 2013). Furthermore, Honnay et al. (2002a) signalises the importance of the connectivity by hedgerows, the size and the shape of the post-agriculture forest speed up the colonisation of forest plants.

While dispersal limitation can be solved, it may be far more difficult to properly identify habitat unsuitability and define management solutions to mitigate it (Tischew and Kirmer 2007; Menges 2008). Menges (2008) related the habitat unsuitability with competitive exclusion of target species by competitors and associated the altered competitiveness with habitat modifications and an unsuitable level of disturbance or stress. Therefore, establishing stress (or disturbance) factors tolerated by AFS might be effective for their restoration on P enriched soils. For example, De Keersmaeker et al. (2004) found that increasing shade in post-agricultural forests counterbalanced the P-altered relation between competitors and AFS. Furthermore, habitat suitability must be seen in the light of all the life stages of plants, including adult growth, flowering, fruiting, seed germination and seedling recruitment, as ontogenetic shifts in habitat requirements regularly occur (Drayton and Primack 2012).

1.3.1 Tree species as ecosystem engineers

Tree species are ecosystem engineers (Jones et al. 1997) that not only determine the wood quantity and quality that will be produced, but also influence all other elements of the ecosystem functioning and ecosystem services. Depending on the species, they create different levels of stress for the understory via their shade and litter effects on soil conditions (Augusto et al. 2003; van Oijen et al. 2005; Barbier et al. 2008; Knight et al. 2008; Tinya et al. 2009; Wulf and Naaf 2009).

This thesis focuses on planted forest where the tree species are selected by the forest manager. Tree species effects can thus be considered as consequences of management choices in contrast to spontaneous afforestations where abiotic conditions and tree species interact. Nevertheless, abiotic conditions and land use history remains important factors and there variation should be controlled to draw valid conclusions on tree species effects. Furthermore, different tree species might also be subjected to differences in management practices, e.g. poplars are planted at a wider spacing than oaks and other broadleaves. In the rest of this thesis, the term tree species effect may be interpreted as the effect of different tree species selected and planted by the forest manager on sites with similar conditions.

Many studies have demonstrated that the dominant tree species determines the light regime, the litter quality and the soil fertility, which subsequently influence the herb layer composition (see Barbier et al. 2008 for an overview). However, the relative importance of these factors and the precise mechanisms behind tree species effects upon the herb layer are still insufficiently known (Barbier et al. 2008; Aerts and Honnay 2011). Moreover, some research has proposed a differential reaction of herb layer species on soil fertility or acidity depending on the light availability or vice versa. Moir (1966), for instance, showed that the growth of a light demanding grass was reduced by soil acidification, but this was more explicit in a shady environment than under full light exposure. Similar interactions have more recently been shown for seedlings of several tree species (e.g. Grubb et al. 1996; Bigelow and Canham 2002; Portsmuth and Niinemets 2007; Palow and Oberbauer 2009).

1.3.2 Soil acidification as tree species effect

Soil acidification by tree species is caused by (1) the time delay between the uptake of base cations in exchange for protons (H^+) and the release of these base cations by the decomposition of the organic matter (Nilsson et al. 1982), (2) the production of organic acids by slowly decomposing litter (Devries and Breeuwsma 1986) and (3) the N_2-fixation by symbiotic bacteria of actinorhizal tree species (Van Miegroet and Cole 1984). N_2-fixation is limited to only a few tree species found in northwestern Europe. The base concentration (mainly Ca) in the litter is the main source of base cation release of trees and thus the main driver of tree species effects on the soil acidification (Reich et al. 2005). Two groups are distinguished: i.e. tree species that release a large proportion of their cation uptake yearly and tree species that withdraw the base cations from the leaves before shedding. Ca concentrations in litter of trees from the first group, e.g. *Tilia cordata* Mill., *Acer pseudoplatanus* L., have been found to be twice as high as in litter from tree species of the second group, e.g. *Quercus rubra* L. and *Carpinus betulus* L. (Binkley and Giardina 1998; Reich et al. 2005). In the first group, the high yearly release of Ca will prevent the top soils from acidifying. The Ca retention by the trees of the second group, results in a declining soil pH, exchangeable Ca and base saturation and increasing C content and C/N ratio (Reich et al. 2005; De Schrijver et al. 2012a). Soil acidification and increasing aluminium (Al) availability in the soil solution result in decreasing earthworm abundance and diversity. Because earthworms are keystone species for litter decomposition, decreasing earthworm populations will lead to further litter accumulation and soil acidification (Muys and Granval 1997; Reich et al. 2005; Edwards et al. 2009). At the same time, the litter decomposers will shift from macro-invertebrates and bacteria to fungi, resulting in an increased release of organic acids.

The change of the soil acidity is buffered by various mechanisms. With increasing input of H^+, first carbonate will dissolve, secondly cations on the soil complex will be exchanged against H^+ and thirdly Al and, finally, Fe will be exchanged against H^+ (Fisher and Binkley 2000). Once the soil acidity has reached the Al buffer range (around pH-H_2O of 4.2), increasing amounts of Al will become bio-available which leads to Al toxicity and nutrient imbalances in some plants (Cronan and Grigal 1995; Weber-Blaschke et al. 2002). Acid intolerant tree species are found to suffer from Al accumulation within the root tissues and Ca, magnesium (Mg) and/or P deficiency due to Al antagonism (Weber-Blaschke et al. 2002). Further, acid intolerant species are also more Ca and Mg depending than other species (Falkengren-Grerup 1995; Lucash et al. 2012). In contrast, plant species that are adapted to grow in more acidic soils show stress avoidance or tolerance strategies (Andersson 1988).

Besides chemical effects of soil acidification also mechanical effects of litter accumulation are a factor in the tree species effect. However, litter accumulation is correlated with soil acidification and consequently often studied together. Experiments have provided evidence that litter may alter growth and biomass production of species in different ways (Sydes and Grime 1981a). In general forest grasses show reduced growth when litter depth increases while typical forest perennials, like *Hyacinthoides non-scripta* (L.) Chouard ex Rothm., *Anemone nemorosa* L. and *Lamium galeobdolon* (L.) L. are more insensitive. As a consequence, the competitive relations between both groups are affected (Sydes and Grime 1981b). Staaf (1992) even found negative effects of litter removal on introduced adult AFS plants. In contrast, litter removal has beneficial effects for seedling recruitment for most forest plants (Eriksson and Ehrlén 1992; Eriksson 199). None of the species investigated by Eriksson (1995) was favoured by litter, *A. nemorosa* was one of the few species capable to germinate in litter. Leaf litter thus might have both beneficial and negative effects: for the initial germination and recruitment little litter is tolerated while in later stages some litter might be helpful to give competitive advantages to forest perennials as long as they can cope with the associated soil acidity.

The relevance of tree species effects on soil acidification will strongly depend on the soil type. Sandy soils (e.g. in the Campine region) with little buffering capacity against acidification will be strongly acid, irrespective of the tree species planted. Consequently, most of the forested sandy soils will shift to the Al buffer range and acid intolerant species will be absent. On the other hand, soils that are rich in free Ca, e.g. in sites where base rich source water constantly resets the acidification or on heavy clay soils with a high CEC and base saturation (e.g. in the polders), the tree species will have little effect on the soil acidity (e.g. van Oijen et al. 2005; Kooijman 2010) which remain independently high. In contrast, tree species have very strong impacts on moderately buffered soils (van Oijen et al. 2005), like the loam and sand loam soils in Flanders (Brahy et al. 2000).

Finally, soil acidity also affects the bio-availability of P, both physico-chemically (Stevenson and Cole 1999) and biologically via P solubilisation by soil organisms (Bolan

1991; Gyaneshwar et al. 2002). This is of potential importance since high soil P concentrations of post-agricultural forests are strongly limiting the herb layer development.

1.3.3 Shade casting as tree species effect

Differences in light transmittance of tree species is related to differences in canopy structuring elements, often summarized in Leaf Area Index (LAI). The LAI depends on the leaf size, spatial density and orientation of the leaves and light captation capacity of the leaves. In their review, Barbier et al. (2008) found some differences between tree species, for example *Fagus* sp. having lower light transmittance than *Betula* sp., but a detailed list of species ordered according to their light transmittance could not be presented due to variation among studies.

This may partly be explained by the difficulty of getting a good estimate of the light transmittance, since incoming light above the forest stands differs in intensity and direction throughout the day, the season and with changing cloud cover (Lieffers et al. 1999). Also the foliage cover differs throughout the season and varying canopy phenologies may effect AFS differently (e.g. Landhäusser et al. 1997; Rothstein and Zak 2001; Kudo et al. 2008). For this purpose many techniques have been developed and evaluated: radiometers, photosensitive chemicals, hemispherical canopy photographs, plant canopy analysers or visual estimates (Lieffers et al. 1999). However, irrespective of the light measurement used, responses of the understory to different amounts of light are sometimes not captured (e.g. Augusto et al. 2003). Furthermore, it has been suggested that vegetation response might better fit with the past light regime than the current because of slow shifts in species composition (Thomas et al. 1999).

1.3.4 Other tree species effects

Understory light availability is correlated with temperature and humidity regimes at the forest floor. Consequently, understory light should be treated as a synthetic factor grouping all these effects (Barbier et al. 2008). Root competition for water between tree and herb layer may be an additional tree species effect but no studies on this subject have been published (Barbier et al. 2008).

N_2-fixing tree species can create elevated soil N concentrations (Van Miegroet and Cole 1984). Elevated N concentrations were found to improve biomass production of only few forest herbs and half of the forest grasses studied by Falkengren-Grerup et al. (1998), while very high N concentrations negatively affected most species. Consequently, N_2-

fixing tree species are richer in nitrophilous species of the understory (Dzwonko and Loster 1997).

Finally, phytotoxic chemicals can be released by tree species which may affect some understory species (Mallik 2008). These can originate from throughfall water, litter decomposition and root releases. For example, Kill (1992) proved that extracts of needles of different *Pinus* sp. inhibited seed germination of various understory species explaining the impoverishing effect of *Pinus* sp. on the herb layer species richness compared to other tree species which he observed.

Summarizing, tree species effects on the herb layer can be explained as a combination of differences in light transmittance, microclimate, soil nutrients and acidification, physical effects of the litter, root competition, N_2-fixation and release of phytotoxic chemicals.

1.3.5 Tree species effects in post-agricultural forests

Post-agricultural forests have environmental characteristics that change over time (e.g. Bellemare et al. 2002; Brunet et al. 2011). Therefore, tree species effects on the post-agricultural forests ecosystem are likely to diverge in time (Barbier et al. 2008; De Schrijver et al. 2012a) and conclusions from studies in long-established or ancient forest are not directly transferable to post-agricultural forests. In post-agricultural forests, soil acidification may be even more profound since these soils were regularly limed and often have a near neutral soil pH at the time of afforestation. Some tree species show a rapidly declining pH, in contrast to tree species that show only limited acidification (De Schrijver et al. 2012a; Sauer et al. 2012). However, tree species effects on recruitment of AFS in post-agricultural forests have hardly been studied (but see Dzwonko and Loster 1997; Ritter et al. 2003; Wulf and Heinken 2008). These three studies found differences in the herb layer between post-agricultural coniferous and deciduous forests. Pensa et al. (2008), finally, found evidence that different tree species can create environmental limitations for the development of typical forest perennials in recent forests on mine spoil.

1.3.6 Tree species used in Flemish post-agricultural forests

In Flanders, about 18280 ha were afforested between 1910-1940 and 2000 on loam and sand loam soils (De Keersmaeker 2013). Based on the forest map (AGIV 2001), 91% of this forest is broadleaved. At least 72% of these broadleaved stands are homogenous stands, including 66% *Populus* spp., 5% *Quercus* spp. and 2% *Fagus sylvatica* L.. The remaining 28% includes both homogenous stands with other broadleaved species and mixed stands. The coniferous stands include in decreasing order *Picea abies* (Karst.) L., *Pinus sylvestris* L., *Larix* spp. and *Pinus nigra* Arnold.

To study the remaining tree species in more detail, two other data sources can be used. Between 2003 and 2013, the Flemish Agency for Nature and Forests, approved subsidies for the afforestation of 644 ha of agricultural land (ANB 2013). On 50% of the area *Q. robur* was planted, *Fraxinus excelsior* L. 15%, hybrid poplars without shrubs 11% and with shrubs 6%, *Alnus glutinosa* (L.) Gaertn. 5%, *F. sylvatica* and *Quercus petraea* Lieblein each 2%, *Betula* spp., *Prunus avium* (L.) L., *C. betulus* and *Tilia* spp. each 1%. The remaining 3% is distributed over 13 other tree species. Note that there are no subsidies for non-native trees like *Q. rubra* and that the subsidy varies from 3700€ /ha for native oaks to 850€ /ha for hybrid poplars without shrubs. The forest inventory (Waterinckx and Roelandt 2001) includes 77 plots of afforestations between 1948-1967 and 1997-1999 of arable land or grassland on loam and sand loam soils. In 65% of the cases, *Populus* spp. was the dominant tree species (highest basal area), followed by *Salix* spp. (12%), *Q. robur* (8%), *Betula* spp. (4%) and eight other tree species (<4%).

It can be concluded that on about half of the post-agricultural forests on loam and sand loam, hybrid poplars are planted. *Q. robur* is the second most planted tree species, followed by *F. excelsior*, *F. sylvatica* and *A. glutinosa*. Furthermore, on some percentages of the surface, spontaneous colonising tree species are (temporarily) dominant (e.g. *Salix* spp. and *Betula* spp.).

1.4 Objectives and outline of the thesis

1.4.1 Objectives

Agricultural land has been afforested widely throughout Europe and elsewhere in the world. While in the past the main focus was to provide production forest, a shift is taking place to focus on ecological and recreational objectives. At the same time, land used for afforestation shifted from mainly marginal land to well fertilized rich soils. However, success of ecological restoration on these sites is limited by the dispersal and environmental limitations of AFS. Environmental limitation of these species has been explained as a consequence of increased competition by P enrichment (Baeten et al. 2009b).

Applying the appropriate level of stress or disturbance has been suggested to improve the success of restoring respectively stress and disturbance tolerant communities. This might be especially important on P enriched sites. Most of the AFS are to some degree shade and litter tolerant, some are acid tolerant but most species are acid intolerant. Therefore, regulating the appropriate level of shade might facilitate the colonisation of AFS in post-agricultural forests, while the high soil pH of former agricultural land might be appropriate to restore both acid intolerant as tolerant species.

Tree species can be seen as ecosystem engineers that mainly determine the ecosystem functioning and biogeochemical cycling of the forest. Tree species strongly differ in shade casting ability, litter accumulation and soil acidification, which on its turn influences the herb layer. Consequently, different tree species might have diverging effects on the colonisation of AFS in post-agricultural forests. However, tree species effects on the herb layer in post-agricultural forests are poorly studied. Probably, increasing shade will suppress competitors and improve the colonisation of AFS and acidification will exclude acid intolerant species.

The research questions of this thesis are:

1. Whether tree species differentially influence the colonisation of AFS in post-agricultural forests?
2. How tree species effects vary with the age of the afforestation?
3. How tree species effects vary with different life stages of the AFS?
4. How tree species effects on the colonisation of AFS can be explained?
5. How tree species effects can be used as a steering mechanism for the ecological restoration of post-agricultural forests?

This thesis is limited to well fertilized post-agricultural forests on rich loam and sand loam soils. The loam and sand loam soil types have particularly low (ancient) forest cover in Flanders and elsewhere and are the richest in AFS, making them a priority for ecological restoration. Furthermore, tree species effects are expected to be most profound on these soils. Furthermore, I will focus on post Second World War afforestations since these have the most strongly increased P concentrations.

1.4.2 Outline

After this introduction (Chapter 1), 5 chapters follow, each presenting the results of separate experiments or vegetation recordings (Chapter 2-6). Thereafter, a comprehensive discussion and conclusions (Chapter 7) is given (Figure 1.1).

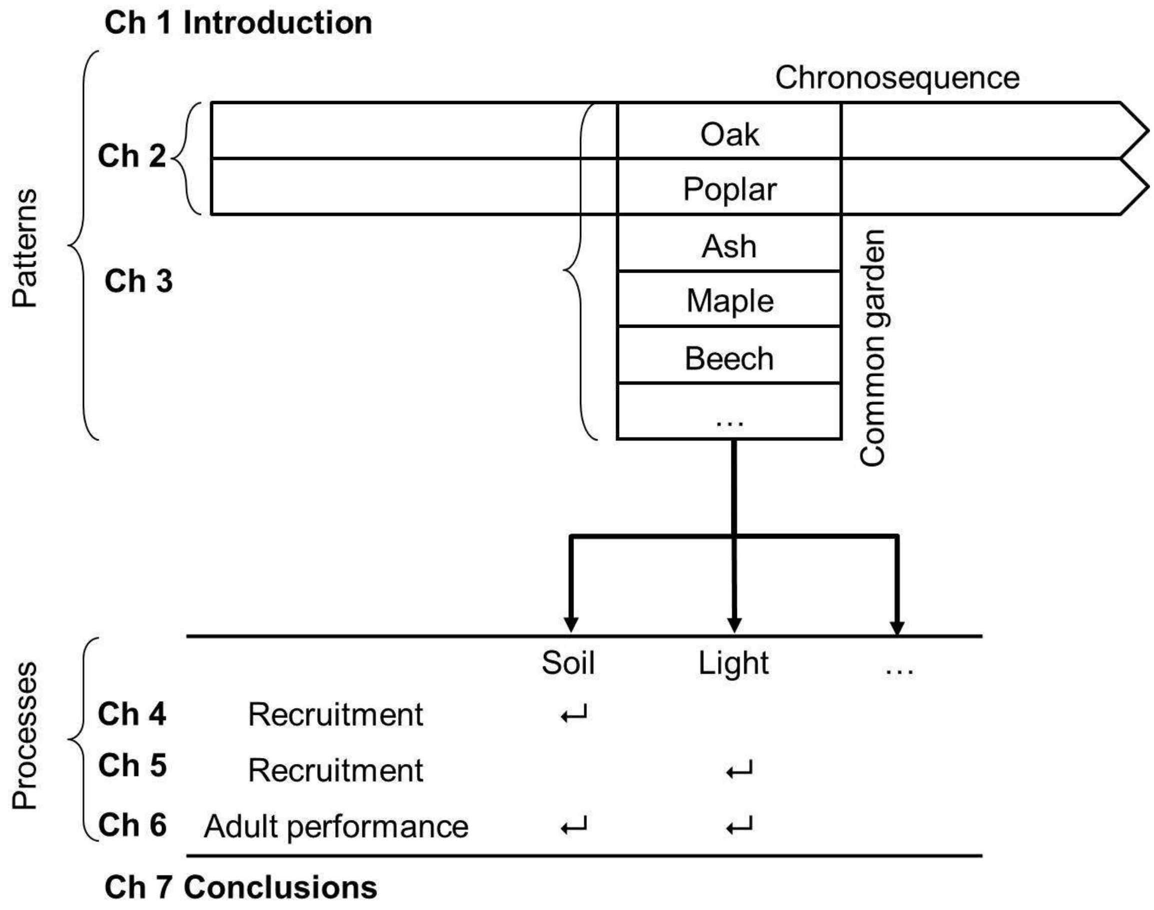

Figure 1.1 Outline of the thesis: chapter 2 and 3 focus on the general vegetation patterns of, respectively, a chronosequence with two tree species and a common garden with eight species whereas chapter 4, 5 and 6 comprise experiments that focus on specific processes on different life stages.

Chapter 2 examines the patterns of vegetation development under poplar and oak, two contrasting tree species for light transmittance and litter quality which are most often used for afforestations in Flanders. Within this chronosequence study, the divergent vegetation development between these two species is studied. The 3th chapter compares a larger set of eight tree species. An introduction experiment was set up in a common garden (see 1.4.3 Mortagne forest as research site) and monitored for 9 yr. Here the focus lies upon AFS performance under different tree species and the main factors explaining the tree species effects. Thereafter, results of three experiments are reported that aim at further unravelling the tree species effect by studying one or two underlying factors, explaining the tree species effect. The first, in chapter 4, is based on an ex situ experiment and aims to study tree species effects through their impact on the soil on the germination and recruitment of AFS. Chapter 5 looks at the effect of difference in leafing phenology of canopy shrub species. Here the first results of this experiment are presented. Chapter 6 examines a potential interaction between light availability and soil acidification and, furthermore, tries to link the performance of AFS with the soil acidification via the plant nutrient status. In chapter 7, finally, an overall discussion is presented and here I will present a simple framework to explain tree species effects on the herb layer.

1.4.3 Mortagne forest as research site

Mortagne forest (50.77° N, 3.37° E, 30-40 m a.s.l., Belgium) is a post-agricultural forest of about 16 ha which was created in 1972. The forest lies in Zwevegem, near Kortrijk, and is managed by the Flemish Agency for Nature and Forests.

The forest has some unique features which make it an ideal location for research on tree species effects in post-agricultural forests.

1) The forest is strongly isolated form other forests and most forest plant species were absent prior to the introduction (chapter 3). The Mortagne forest is mainly surrounded by arable land (Figure 1.2). In recent years, efforts are made to connect the Mortagne forest to the Orveyt forest, which lies one km northeast of Mortagne forest. Orveyt forest has a small piece of forest that might originate from before the Ferraris map (1771-1775), the rest was afforested in 1988-1990. The nearest ancient forests are Beer forest and Grandval-Kooigem forest which, respectively, are located 2 and 2.5 km away from Mortagne forest.

2) The different stands of the forest have a very similar soil. The soil of this forest is a well-drained quaternary niveo-eolian loam soil, classified as a Haplic Luvisol (IUSS Working Group WRB 2006). This soil type in particular is very sensitive for acidification, either caused by natural or anthropogenic factors (Brahy et al. 2000). Under forest, the level of soil acidification of this soil type is determined by tree species (Neirynck et al. 2000; van Oijen et al. 2005).

3) The different stands of the forest have a very similar land use history. On old topographical maps (1771-1775, 1846-1854, 1862 and 1910) the area is shown as arable (1771-1775 does not distinguish between grassland and arable). According to aerial photos and a topographical map of 1952 and 1959, the area was used as grassland with the exception of stands Fr1, Ti4 and Ac4 (Figure 1.2), which remained arable. When afforested in 1972, the area was used as arable again except a piece that was still grassland (Figure 1.2). On older topographical maps the private forest parcel had a farm, while it was arable in 1910 and forest from 1952 onwards (Thomaes and Vandekerkhove 2005).

Figure 1.2 Stands of Mortagne forest on an aerial photo of 2012. Stand names (Table 1.1) indicate stands that originate from 1972. pri: private forest stand. Dotted lines: historic parcel borders and green shaded area: grassland before afforestation.

4) Different commonly used broadleaved tree species are present in homogeneous stands of the same age. In total, 18 stands originate from 1972 and were planted with 13 different tree species (Figure 1.2, Table 1.1). Some other stands were planted more recently.

In most of the next chapters (except chapter 5) experiments will be performed (partly) in Mortagne forest or with soil collected in this forest. Previous research was performed by Dossche (1998) and Verstraeten et al. (2004). Dossche (1998) studied the tree species effects on the soil development by examining the amount of litter, different litter nutrient concentrations and soil nutrient concentrations (Figure 1.3). pH-KCl clearly differed between tree species for the topsoil (0-5cm). The highest topsoil pH-KCl was found in the two *Populus* stands, followed by *Fraxinus*, *Tilia* and *Prunus*, which again differed from *Q. robur*, *Acer*, *Fagus* and *Alnus*. At the depth of 20-40 cm, only the pH-KCl of *Acer* was clearly lower than all other species (Dossche 1998). The fact that the soil acidity is less pronounced at greater depths, suggests that the differences in topsoil acidity are mainly explained by tree species and less likely by differences in land use history or soil type. Verstraeten et al. (2004), furthermore, analysed the soil textures of the samples taken by Dossche and concluded that there is little difference between stands and different depths (0-5, 5-10, 10-20, 20-40 cm). From the vegetation survey, Verstraeten et al. (2004)

concluded that there is a clear negative relation between the cover of the tree and shrub layer with the cover of the herb layer and shade intolerant species in particular. The most light rich conditions were found under *Salix caprea* L., *P. avium*, *Fraxinus americana* L. and *Robinia pseudoacacia* L., while the darkest conditions were underneath *Q. rubra, F. sylvatica*, *A. pseudoplatanus* and *T. cordata.* The two stands with *Populus* x *canadensis* Moench and the stand with *A. glutinosa we*re intermediate at that time.

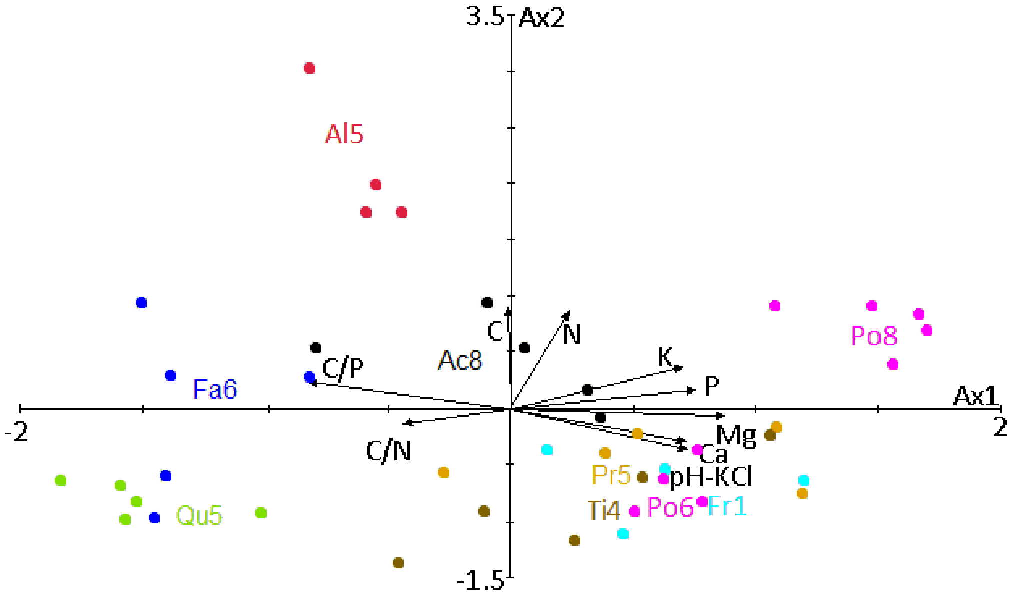

Figure 1.3 PCA ordination based on soil analyses of 9 stands (Table 1.1) of Mortagne forest (redrawn from Dossche 1998)

Table 1.1 Description of the stands originating from 1972 including stem density and basal area (measured in 2003, Thomaes and Vandekerkhove 2005)

Stand	Tree species	Shrub layer	Stand changes	Stem density (ha^{-1})	Basal area (m^2.ha^{-1})
Fr1	*Fraxinus americana*	Spontaneous recruiting trees		1456	30
Al2 + So2	*Alnus glutinosa* and *Sorbus aucuparia* L.	Spontaneous (*Sambucus nigra* L.)		1574	36
Be3	*Betula pubescens* Ehrh.	None		1454	22
Fr3	*Fraxinus americana*	Spontaneous recruiting trees		1454	32
Ti4	*Tilia cordata*	None	2012: partly coppiced	2240	40
Ac4	*Acer pseudoplatanus*	None		1218	29
Ti5	*Tilia cordata*	None		2243	41
Al5	*Alnus glutinosa*	Spontaneous (*Sambucus nigra*)		1100	25
Pr5	*Prunus avium*	None		1101	28
Sa5	*Salix caprea*	None		2045	19
Ro5	*Robinia pseudoacacia*	Spontaneous (*Sambucus nigra* and *Coryllus avellana* L.)		511	24
Qu5	*Quercus robur*	Spontaneous (*Sambucus nigra)*		708	36
Qr6	*Quercus rubra*	Spontaneous recruiting trees		1495	32
Po6	*Populus* x *canadensis* ‘Harff’	*Sorbus aucuparia* and *Coryllus avellana*	2004: clear cut, replanted with *Fraxinus excelsior*	177	50
Fa6	*Fagus sylvatica*	None		1062	29
Ac8	*Acer pseudoplatanus*	Spontaneous (*Sambucus nigra*)		786	22
Po8	*Populus* x *canadensis* ‘Robusta’	*Castanea sativa* Mill.	2008: clear cut, replanted with *Fraxinus excelsior*	118	27

2

Diverging herb layer development in a chronosequence of post-agricultural forests with two contrasting tree species

After: Thomaes, A., De Keersmaeker, L., Van Calster, H., De Schrijver, A., Vandekerkhove, K., Verstraeten, G. & Verheyen, K. 2012. Diverging effects of two contrasting tree species on soil and herb layer development in a chronosequence of post-agricultural forest. Forest Ecology and Management, 278: 90-100

Abstratct

The restoration of forest ecosystems on former agricultural land faces numerous problems. Recolonisation of forest species is hampered by the modified habitat quality and by the isolation from source populations. Tree species are ecosystem engineers that can modify soil and light conditions and can, therefore, act as a possible catalyst for understory recovery. Therefore, I set out to study the effects of tree species on the herb layer development. For this purpose, a chronosequence of post-agricultural oak (*Q. robur* and *Q. petraea*) and poplar (*P. x canadensis* and *Populus x generosa* A. Henry) plantations on loam and sand loam soils was selected. The selected tree species are frequently planted and have contrasting characteristics (e.g. in terms of litter quality, shade casting ability and growth rate). Under plantations of *Quercus*, soils acidified with increasing stand age and dropped into the Al buffer range after only 20 to 30 years, whereas soil pH-H_2O hardly changed under *Populus* plantations. Soil C and N content increased with stand age, while C/N ratio depended on tree species but remained constant in time. Multivariate analysis revealed that vegetation development depended on tree species, isolation and stand age. *Populus* stands were characterised by rough grassland species while *Quercus* stands had low understory cover and consequently had no indicator herb species. No tree species effect was found on the cover of strict forest species and AFS. *Quercus* favoured

acid tolerant forest species, while *Populus* favoured light demanding and acid intolerant forest species. Our results lead to the conclusion that tree species can be important drivers of vegetation development in post-agricultural forests, in particular through their impact on soil development and light availability for the understory. Therefore, it is important that goals for soil development and understory restoration are taken into account when tree species are selected for afforestations. Acidifying tree species may have irreversible effects, permanently excluding acid intolerant species. This is especially important when aiming to safeguard acid intolerant forest species from acidification in ancient forest by creating post-agricultural forest as refugee zones. When a species rich herb layer with acid intolerant species are aimed at, selecting a tree species with a good decomposable litter like *Populus* is a necessity and suppressing competitors by a shady overstory of the tree or shrub layer. Soil acidifying species like *Quercus* might be more suitable to enlarge forest habitats that mainly contain acid and shade tolerant species.

2.1 Introduction

Forest restoration receives increasing attention in aiming to save forest biodiversity and to safeguard ecosystem services suffering from deforestation, degradation and climate change (Aerts and Honnay 2011; Holl and Aide 2011). Areas with a low and fragmented forest cover in particular should include afforestation efforts into their restoration projects to realise their goals (Hartley 2002; Stanturf and Madsen 2002). On that account, fertile farmland has been converted to forest in the temperate zones (e.g. Vellend 2003; Hermy and Verheyen 2007; Zhang et al. 2010; Brunet et al. 2011). Habitat restoration on former farmland is, however, strongly limited by the slow colonisation of AFS (Hermy et al. 1999; Verheyen et al. 2003a), which are often indicator species of the aimed forest habitat.

The limited colonisation of post-agricultural forest by AFS has been explained by a combination of dispersal and environmental limitation (e.g. Verheyen et al. 2003a; Baeten et al. 2009b). This Environmental limitation of AFS in post-agricultural forests (i.e. recent forest on fertile former farmland) is mainly assigned to legacies of the past land use (Koerner et al. 1997; Bossuyt et al. 1999b; Verheyen et al. 2003a; Verheyen and Hermy 2004; Baeten et al. 2009b). Post-agricultural forests soils are mostly differentiated from ancient forest soils by elevated soil pH and P concentrations (Koerner et al. 1997; Dupouey et al. 2002; Vesterdal et al. 2002; Hooker and Compton 2003; Ritter et al. 2003; Hermy and Verheyen 2007; Zhang et al. 2010; De Schrijver et al. 2012b). De Schrijver et al. (2012b) demonstrated that the bio-available P fraction decreased with stand age in

Quercus afforestations, except in the 0-5 cm of topsoil, where this labile P fraction remained persistently high. The persistently increased bio-P is suggested to be one of the main reasons for a failing recruitment (Koerner et al. 1997; Verheyen et al. 1999; De Keersmaeker et al. 2004). For example Baeten et al. (2009b) found the recruitment, longevity and performance of an AFS to be lower in post-agricultural forests with a ten times higher bio-P, than in unfertilized ancient forest. Forest herbs generally benefit directly from the increased bio-availability of P in post-agricultural forests (Baeten et al. 2010). However, since an increased bio-P promotes P-opportunistic competitors, like *U. dioica*, even more, overall effects are negative by enhanced aboveground and belowground competition (Baeten et al. 2009b).

Management adaptations, like raising the canopy cover to limit the growth of competitors (De Keersmaeker et al. 2004) or selection of a tree species that can decrease P-availability, have received little attention. Tree species could be used as ecosystem engineers that promote understory recovery in post-agricultural forests (Dzwonko and Loster 1997; Wulf and Heinken 2008; chapter 1). Several studies indicate that tree species can influence herb layer composition, with the light regime and litter quality as main drivers (e.g. van Oijen et al. 2005; Barbier et al. 2008; Van Calster et al. 2008; Wulf and Naaf 2009; Kooijman and Cammeraat 2010). Therefore, I suppose that tree species could also be used as a catalyst for the understory recovery in post-agricultural forests. However, most overstory-understory studies have been performed in ancient forests and in fully grown stands (Aubert et al. 2004; van Oijen et al. 2005; Van Calster et al. 2008; Wulf and Naaf 2009; Kooijman and Cammeraat 2010; but see Dzwonko & Loster 1997; Ritter et al. 2003). In contrast, developing, post-agricultural forests have bio-environmental characteristics that change over time (e.g. Bellemare et al. 2002; Brunet et al. 2011). Therefore, tree species effects on the post-agricultural forests ecosystem are likely to diverge in time (Barbier et al. 2008) and conclusions from studies in long-established or ancient forest are not directly transferable to post-agriculture forest.

Our research aimed at studying the effects of two tree species on the vegetation composition in post-agricultural forest via their effects on environmental variables, in relation to time after afforestation. For this purpose, I selected a chronosequence (space for time substitution) of stands with two frequently used tree species with contrasting characteristics on well drained loamy soils which are vulnerable for acidification (Van Calster et al. 2007; Baeten et al. 2009a; De Schrijver et al. 2012a). This soil type potentially harbours a high number of AFS, but forest cover has been strongly reduced on these sites in Flanders (De Keersmaeker et al. 2013). This makes them of focal interest for restoration purposes.

2.2 Material & Methods

2.2.1 Study design

In northwestern Belgium 64 stands were selected based on the following criteria: (1) with 1st generation of even aged trees of 5-40 yr old on former agricultural land, (2) on well drained loam or sand loamy soil (Haplic Albeluvisols and Haplic Luvisols), (3) either adjacent to an ancient forest (n= 45) or isolated from ancient forests and other sources with populations of AFS (n= 19) and (4) the dominant tree species were either native oaks (*Q. robur* and *Q. petraea*; n= 36) or poplar cultivars (*P.* x *canadensis* and *P.* x *generosa*; n= 28, Figure 2.1). *Populus* are fast growing trees with an open canopy and well decomposable litter. In contrast, *Quercus* grow slower, have a shadier canopy and more slowly decomposing litter that accumulates on the forest floor and acidifies the soil. Poplars are planted as poles in a grid of 8x8 m up to 10x10 m. Poplars are planted in homogenous stands, in half of the cases they had a planted or spontaneous shrub layer (in 49% the sum of shrub layer cover >50%). The *Quercus* stands are planted in a grid of 1x1 m up to 2x2 m, followed by gradual thinning. In two occasions the oaks were sown. In some occasions Oak stands were mixed, but plots for vegetation survey were positioned under homogeneous blocks of oak. Except, in three very young stands *S. caprea* or *Betula pendula* Roth covered about 30% and in five other stands secondary canopy species covered up to 30% of the plot. Stands adjacent to ancient forest have at most a small ditch or unpaved road in between, while isolated stands are located at least 200 m, mostly several km, from ancient forest. Whenever possible, neighbouring stands with *Quercus* and *Populus* with similar age and soil were included (n=10x2). I tried to select a balanced set of stands for all mentioned criteria (see Results and Table 2.1). For each stand, I determined the land use prior to planting (grassland or cropland) and the year of conversion to forest based on data of the owner, Flemish Agency for Nature and Forests, previous research or tree ring analyses. All stands were converted from farmland to forest between 1965 and 1999. The area of the selected stands ranged from 0.07 to 3 ha.

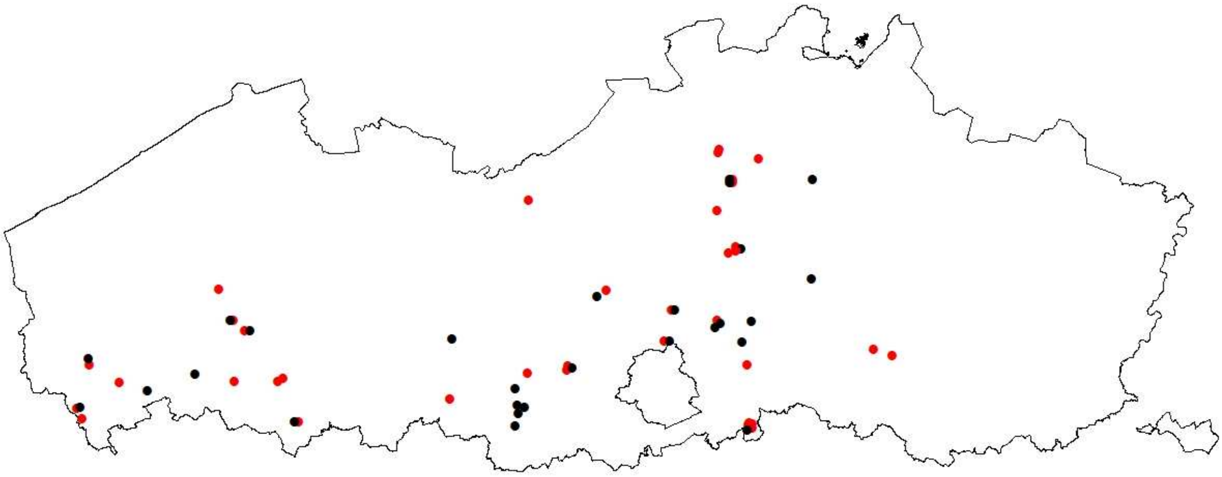

Figure 2.1 Location of the 64 plots within Flanders. Black circles: *Populus* plots and red circles: *Quercus* plots

The agriculture in the study region is very intensive, both on crop field and grasslands that both receive yearly dunging and regularly liming. Furthermore, many of the selected sites shifted from crop field to grassland in the 20th century prior to afforestation. Based on 6 historical maps (1865, 1890, 1909-1940, 1960 and 1980) 21 (70%) of the sites planted on crop fields were permanent crop fields while only 3 (10%) sites were on permanent grassland and 3 others were grassland between deforestation and afforestation. The soils in the study region that are in agricultural use are very rich in bio-P (extracted with ammonium lactate), cf. Boon et al. (2009) more than 80% of the fields have more than 130 mg P.kg^{-1} with little difference between three agricultural regions (Flemish sand region, sand loam region and loam region), crop field and grassland and period of analyses (1989-1991 to 2004-2007). Agricultural fields have near neutral pH both in grasslands and crop fields, cf. Boon et al. (2009) more than 90% of fields have a pH-KCl of more than 4.5 (>73% have more than 5.1). Post-agricultural forests in the study region resulted mainly from a policy to increase the forest cover and to a lesser account from land abandonment for which site selection depends on soil conditions.

2.2.2 Vegetation survey

To assess the vegetation composition, the cover of each species was recorded using the Londo scale (Londo 1976) in plots of 16x16 m. The cover of woody species was subdivided in seedlings of <0.5 m (included in herb layer), <6 m (shrub layer) and larger individuals (tree layer). When placing the plots, ditches were avoided, disturbed soils, forest edges and other non-representative areas. In stands adjacent to ancient forest, plots were located 16 m away from the border with the ancient forest. Data collection took place in 2005, 2006 and 2008 and vegetation descriptions in each plot were made both during spring (May) and summer (late June to early September). For each species and layer the highest cover from spring and summer data was used for further analyses.

Table 2.1 Percentage (%) of the stands within the study setup divided by stand age and isolation (a) and sand fraction and former land use (b). In (a) the numbers before the dash (/) represents stands where a deeper soil sample was collected and after the dash stands without deeper soil sample.

TS	*Populus*		*Quercus*		
Isolation	Adj	Iso	Adj	Iso	Total
(a)					
Age: 5-10 yr	0/0	2/2	2/6	0/6	17
11-20 yr	6/11	0/3	8/13	3/2	48
21-30 yr	3/2	0/5	3/8	0/3	23
31-40 yr	0/5	32/0	0/2	2/0	11
Total	30	14	41	16	
	44		56		

TS	*Populus*			*Quercus*			
FL	Cr	Gr	NA	Cr	Gr	NA	Total
(b)							
%S: 10-19%	3	5	0	3	2	2	14
20-29%	3	8	0	6	5	0	22
30-39%	0	3	0	6	3	2	14
40-49%	2	3	2	2	2	0	9
50-59%	0	6	0	3	2	0	11
60-69%	5	0	0	6	3	0	14
70-79%	2	3	0	6	5	0	16
Total	14	28	2	33	20	3	
	44			56			

TS: Tree species; Adj: Adjacent to ancient forest; Iso: Isolated from ancient forest; FL: Former land use with Cr: Crop field and Gr: Grassland and %S: Sand fraction.

2.2.3 Soil sampling and analyses

Soil samples were collected during the summer inventory at a depth of 0-10 cm in the A horizon (further referred to as mineral topsoil), as a mixture of samples from 3 (2008) or 4 (2005) places at the border of the vegetation plot. In a subset of 20 plots (Table 2.1) additional soil samples were collected from a depth of 45-55 cm (further referred to as the deeper soil sample). The deeper soil layers are assumed to be hardly affected by the recent tree species effect (cf. Ritter et al. 2003; De Schrijver et al. 2012a) and, therefore, can be used to evaluate differences in the original soil pH before planting and to validate the chronosequence.

Topsoil samples were analysed for pH-H_2O (ISO 10390 1994), bio-P (Bray and Kurtz 1945) and total P (tot-P; destruction of 0.7 g of soil with 6 ml (37%) HCl and 2 ml (65%) HNO_3 in microwave oven, analysed with ICP), Kjeldahl nitrogen (Kj-N; ISO 11261 1995) and C (as 55% of the loss on ignition between 105 and 550° C) and the deeper soil for pH-H_2O (pH-deep).

Furthermore, the texture from the mineral topsoil was investigated by laser diffraction (Vandecasteele et al. 2002). The particles larger than 50 µm are considered as sand and 6-50 µm as silt. The 0-6 µm fraction from the laser diffraction method correlates with the 0-2 µm clay fraction of the pipette method (Konert and Vandenberge 1997; Vandecasteele and De Vos 2003).

The soil of the selected stands had 9-32% clay, 9-60% silt and 10-80% sand. The fraction of clay was low and correlated with the silt fraction and the amount of silt was negatively related to the sand fraction (Figure 2.2), therefore, only the sand fraction was used for further analyses.

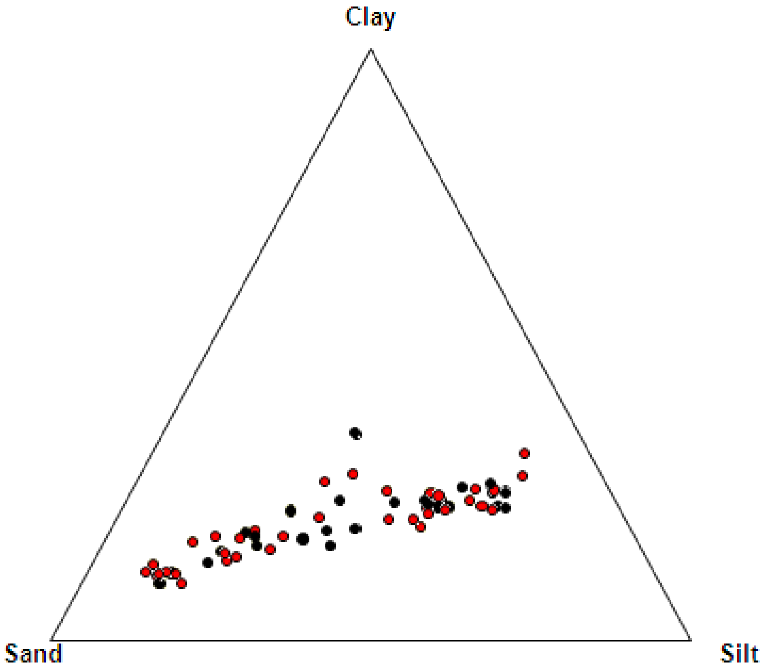

Figure 2.2 Soil texture of the study plots. Black circles: *Populus* plots and red circles: *Quercus* plots

2.2.4 Data analyses

The main explanatory variables used in the analyses are tree species (TS: *Quercus*/*Populus*), stand age (Age) and proximity to ancient forest (Isolation: adjacent/isolated). Former land use (FL: crop/grassland), sand fraction (%S) and the forest complex (Forest; in some cases stands were nested within the same forest) were included as co-variables.

Response variables were either environmental variables or vegetation characteristics. The selected environmental variables are those that can be influenced by tree species and are potential drivers of understory vegetation development: the overstory cover (%Overstory; combined cover of the trees and shrubs) and soil variables (pH-H_2O, bio-P, Kj-N, C and C/N). The vegetation-derived variables were based on the herb layer data (excluding tree and shrub seedlings), since herb layer species are generally not deliberately introduced, in contrast to trees and shrubs: (1) total cover (%Understory), (2) the cover of forest species (list from Honnay et al. 1998 annex 1) all together (FS) and subdivided in cover of AFS (list from De Keersmaeker et al. 2011a), acid tolerant species (acidFS; mean R of Ellenberg et al. (1992) and Hill et al. (1999) < 6.5; Box 2), acid intolerant species (acidintolFS; R$\geq$6.5), light demanding edge species (LightFS, Honnay et al. 1998 annex 1) and shade tolerant species (shadeFS; FS-lightFS) and, (3) CSR signature (Hunt et al. 2004) and (4) mL, mR, mN based on the mean value of Ellenberg et al. (1992) and Hill et al. (1999) for each species. This is in accordance with the intermediate geographical position of the study area (cf. De Keersmaeker et al. 2011b). CSR signature and mL, mR and mN were calculated as weighted averages based on the cover of each species. From the CSR signature, the S and C fraction (S-herb; C-herb) was analysed since R values were very low and rather constant. Furthermore some validation data (pH-deep, tot-P and %S) was analysed to validate the stands in the chronosequence. This is especially important in a chronosequence study, since the space for time substitution is only valid if the selected plots originally had similar site conditions.

First, the effect on community composition was modelled with a PERMANOVA model (Legendre and Anderson 1999; Anderson 2001; McArdle and Anderson 2001) as implemented in the vegan package of R (Dixon 2003). The model explained the variability in the dissimilarity matrix (using Jaccard distance to calculate distances in species composition between all pairwise combinations of plots) as a function of TS, Age, Isolation, FL and %S. A stepwise selection procedure was used to select the best model. The stepwise procedure retained variables based on highest residual R^2 and a significance level of 5%. The final model was checked for collinearity, which was defined as variables with a variance inflation factor (VIF) larger than 3 (Zuur et al. 2010). Indicator species in the herb layer for the two tree species were evaluated based on Pearson's phi coefficients of association (Chytrý et al. 2002) adjusted for unequal group sizes (Tichý and Chytrý 2006).

Box 2: Ellenberg indicator values

These plant indicator values were created by Ellenberg et al. (1992) giving an indicative value for plant species of central Europe for seven different scales. The indicator values are based on the realized ecological niche and represent the optima of a certain species on an arbitrary scale, for example reflecting to soil acidity but not representing exact mean pH values. Mean indicator values (often weighted with the individual plant cover) of a vegetation can give indices for the ecological conditions of that site. Ellenberg indicator values are expert based, but vegetation data is used to further calibrate the values of species.

Throughout this thesis three of these indicator values are frequently used: i.e. light (L), acidity (R) and fertility (N). Each scale ranges from 1 (plants indicating respectively deep shade, extreme acid soil and extreme infertile soil) to 9 (full sun, calcareous or other high-pH soils, extreme rich soils). Mean Ellenberg values for a site are represented as mL, mR and mN.

Hill et al. (1999) re-calibrated the original Ellenberg indicator values for British plants. As the ecological requirements of species are not always constant in their range, these indicator values differ from the original Ellenberg indicator values.

Secondly, linear mixed models (LMM) were made using backwards selection based on a significance level of 5% explaining the environmental and vegetation characteristics. Besides the main explanatory variables (TS, Log(Age) and I) and co-variables (%S and FL), the interaction for Log(Age) with TS and FL was included. Age was transformed logarithmically to ensure a linear response. Forest was added as a random effect, to account for the nested structure of the data. Isolation was only included for the vegetation characteristics, as it is not expected to have influence on the environmental characteristics. Finally, similar models were made to explain vegetation characteristics by environmental characteristics (%Overstory, pH-H_2O, bio-P, Kj-N, C). 16 plots with an %Overstory less than 80%, including some young stands and *Populus* stands with severe rust-fungus infection, were omitted from models, which include the %Overstory, to ensure a linear relation for %Overstory. Log and square root transformations were performed when needed to obtain a normal distribution. The structure of the models is:

Variable ~ 1 + TS * Log(Age) + I + %S + FL * Log(Age) + (1|Forest)
Variable ~ 1 + %Overstory + pH-H_2O + bio-P + Kj-N + C

Stevenson and Cole (1999) have proposed a theoretical model for the availability of P in relation to soil pH-H_2O, showing two minima for P availability around pH-H_2O values of 5 and 7.5 when P is maximally adsorbed as Al- and Fe-phosphates and Ca-phosphates respectively. A generalised additive model (GAM) model was selected to describe this non-linear relationship between soil pH-H_2O the available P fraction. Square root transformations were performed to obtain a normal distribution. The structure of the model is:

Sqrt(Bio-P /tot-P*100) ~ 1 + s(pH-H_2O)

All data analyses were performed with R 2.11.1 (R Development Core Team 2009).

2.3 Results

The setup was not entirely randomised since most *Populus* stands were planted on former grassland while *Quercus* stands were most often located on former crop field. The pH of the deeper soil, tot-P and sand fraction did not differ between tree species (Table 2.2). Younger stands had a higher tot-P and sand fraction. Tot-P seemed to be correlated with Isolation. Based on this exploratory analysis, sand fraction and former land use were included as variable in the models and the bio-P as well as the fraction (bio-P/tot-P*100) were modelled.

Overall, I found 130 herb layer species, including 18 AFS and 24 other forest species (see Table 2.3 for a list of forest species and their subdivision in ecological groups).

Tree species (TS) and Age were retained as explanatory variables in the PERMANOVA analysis (df 58,1,1; **: $p < 0.01$), with VIF-values near to 1 indicating a good model. Thus, herb layer composition seems relatively unaffected by Isolation, former land use and sand fraction. Figure 2.3 indicates that the tree species has a divergent impact on the vegetation composition over time. With increasing stand age, a gradual shift from rough *Arrhenatherion* grassland (with *Arrhenatherum elatius* (L.) Beauv. ex J. et C. Presl, *Holcus lanatus* L., *Cirsium arvense* (L.) Scop., *Eupatorium cannabinum* L., *Poa trivialis* L. and *Taraxacum* spp. as indicators) to forest vegetation is observed. Older *Populus* plots are characterised by *Hedera helix* L., *Rubus caesius* L., *Glechoma hederacea* L. and *Geum urbanum* L. but *A. elatius* and *U. dioica* remain dominant. By contrast, most of the older *Quercus* plots were favoured by acid tolerant species like *Lonicera periclymenum* L., *Stellaria holostea* L., *Milium effusum* L. and *A. nemorosa*.

Several species were found to be associated with *Populus* stands, mostly species typical for rough *Arrhenatherion* grasslands with a competitive strategy (Table 2.4). The cover of many competitive species was much higher in *Populus* stands, for example *U. dioica* (34±19%), than in *Quercus* stands (15±16%). Only one species categorized as forest species (*Epipactis helleborine* (L.) Crantz), was significantly associated with *Populus.* No species were found associated with *Quercus* stands.

The results of the LMM are presented in Table 2.2 and in Figure 2.4. Tree species influences both environmental variables and the understory vegetation composition. Overstory cover was higher in *Quercus* stands than in *Populus* stands. Mineral topsoil in *Quercus* plots reveals a fast and strong acidification, whereas soil pH-H2O was stable in *Populus* stands (Figure 2.4a).

The bio-P is determined by the total P concentration (Figure 2.4c), resulting from fertilisation before conversion to forest. Total P concentration ranged from 290 to 1660 mg P.kg^{-1} and Bio-P from 1 to 262 mg P.kg^{-1}. Figure 2.4d shows that Bio-P decreases with age and is higher in more sandy soils. The bio-available P fraction decreases with age and the bio-available P fraction is lower in former grassland than in former crop field. A direct tree species effect on the bio-available P fraction was not found. The GAM model that explained the bio-available P fraction by pH-H_2O (intercept: t=12.68, ***: $p < 0.001$; s(pH-H_2O): F=5.41, ***: $p < 0.001$, Figure 2.5), revealed a minimum below a pH-H_2O of 5.

C and N accumulate after conversion to forest but no tree species effect was found. C is lower in more sandy soils while N was higher in former grassland sites. The C/N ratio differed between tree species but remained constant with age. Also the C/N ratio was lower in more sandy soils.

Table 2.2 Parameter estimates of linear mixed models for (a) validation data, (b) environmental and (c,d) vegetation characteristics with baseline set as TS (tree species)= *Populus* stand, I (isolation)= adjacent to ancient forest, FL (former land use)= Crop field.

	df	Intercept	TS	log(Age)	I	%S	FL	TS x log(Age)	FL x log(Age)
(a)									
pH-deep	14	5.9***	-	-	-	-	-	-	-
tot-P (mg.kg^{-1})	38,20,20	1019***	-	-151*	185*	-	-	-	-
%S	39,23	76.7***	-	-12.3*	-	/	-	-	-
FL	36,23	1.1	-1.7*	-	-	-	/	-	/
(b)									
%Overstory>80	31,11	88.9***	3.8***	-	/	-	-	-	-
pH-H_2O	39,19,19,19	4.8***	2.2*	0.3	/	-	-	-1.0**	-
bio-P (mg.kg^{-1})	39,20,20	164**	-	-47**	/	1*	-	-	-
sqrt(bio-P/ tot-P*100)	35,20,20	5.6***	-	-0.8*	/	-	-0.6*	-	-
log(C*100)	37,21,21	0.25	-	0.39**	/	-0.01**	-	-	-
log(Kj-N*100)	35,19,19	1.4***	-	0.5***	/	-	0.2*	-	-
C/N ratio	37,19,19	15.78***	1.51*	-	/	-0.09***	-	-	-
(c)									
%Understory	39,22,22	229***	-98***	-	-	-1*	-	-	-
mN	39,23	6.9***	-0.4*	-	-	-	-	-	-
mL	39,22,22	7.8***	-	-0.7***	0.5**	-	-	-	-
mR	39,21,21,21	6.0***	1.0	0.2	-	-	-	-0.5*	-
sqrt(FS)	39,22,22	-5.3*	-	3.7***	-3.0**	-	-	-	-
sqrt(AFS)	39,22,22	-6.0**	-	3.3***	-2.5**	-	-	-	-
sqrt(shadeFS)	39,22,22	-5.0*	-	3.3***	-2.9**	-	-	-	-

(table2.2 continued)

sqrt(lightFS)	39,22,22	3.69^{***}	-1.19^{*}	-	-	-0.03^{**}	-	-	-
sqrt(acidintolFS)	39,21,21,21	-1.9	-1.8^{*}	2.3^{**}	-2.1^{*}	-	-	-	-
sqrt(acidFS)	39,20,20,20,20	2.1	-8.2^{*}	-0.1	-1.5^{*}	-	-	3.5^{*}	-
S-herb	39,23	0.25^{***}	-	-	-0.09^{*}	-	-	-	-
C-herb	40	0.56^{***}	-	-	-	-	-	-	-

	df	Intercept	%Overstory>80	pH-H_2O	bio-P	Kj-N	C
(d)							
%Understory	36,17	-153NS	-	54^{**}	-	-	-
mN	36,16,16	3.6^{***}	-	0.5^{**}	-	2.3^{*}	-
mL	36,17	5.929^{***}	-	-	0.003^{*}	-	-
mR	36,16,16	4.1^{***}	-	0.4^{***}	-	1.4^{*}	-
sqrt(FS)	36,17	1.0NS	-	-	-	-	1.2^{**}
sqrt(AFS)	36,17	3.90^{***}	-	-	-0.02^{**}	-	-
sqrt(shadeFS)	36,17	4.57^{***}	-	-	-0.02^{**}	-	-
sqrt(lightFS)	36,16,16	-4.81^{*}	-	1.39^{**}	-0.01^{**}	-	-
sqrt(acidintolFS)	36,16,16	-4.38NS	-	1.66^{**}	-0.02^{**}	-	-
sqrt(acidFS)	36,17	9.4^{***}	-	-1.4^{**}	-	-	-
S-herb	36,17	0.7^{***}	-	-0.1^{***}	-	-	-
C-herb	36,17	0.11NS	-	0.08^{**}	-	-	-

TS: Tree species; Age: Age since afforestation; %S: Sand fraction; FL: Former land use; I: Isolation from ancient forest; %Overstory>80: Cover of the tree and shrub layer (restricted to plots with cover > 80%); %Understory: cover of the herb layer; mN, mL, mR: mean Ellenberg values for nitrogen, light and soil acidity respectively; FS, AFS, shadeFS, lightFS, acidintolFS, acidFS: Cover of forest species, ancient forest species, shade tolerant forest species, light demanding forest species, acid intolerant forest species and acid tolerant forest species respectively; S-herb, C-herb: S and C signature (Hunt et al. 2004) of the herb-layer respectively; /: variable not included; -: variable excluded by model selection; NS: not significant; *: $p < 0.05$; **: $p < 0.01$ and ***: $p < 0.001$.

Table 2.3 List of forest species (list from Honnay et al. 1998annex 1) and their subdivision in ecological groups with AFS underlined (list from De Keersmaeker et al. 2011a)

Acid tolerance	Light demanding forest species (Honnay et al. 1998 annex 1)	Shade tolerant forest species (Honnay et al. 1998 annex 1)
Intolerant ($R \geq 6.5$)	*Alliaria petiolata* (Bieb.) Cavara et Grande, *Calamagrostis canescens* (Weber) Roth, *Chaerophyllum temulum* L., *Cirsium oleraceum* (L.) Scop., *Epipactis helleborine*, *Geum urbanum*, *Lapsana communis* L., *Moehringia trinervia* (L.) Clairv., *Stachys sylvatica* L.	*Adoxa moschatellina* L., *Arum maculatum* L., *Circaea lutetiana* L., *Hedera helix*, *Lamium galeobdolon*, *Listera ovata* (L.) R. Brown, *Paris quadrifolia* L., *Polygonatum multiflorum* (L.) All., *Primula elatior* (L.) Hill, *Ranunculus ficaria* L.
Unspecified		*Scrophularia nodosa* L.
Tolerant ($R < 6.5$)	*Ajuga reptans* L., *Carex pallescens* L., *Epilobium angustifolium* L., *Fragaria vesca* L., *Rubus idaeus* L., *Vicia sepium* L.	*Anemone nemorosa*, *Athyrium filix-femina* (L.) Roth, *Carex remota* Jusl. ex L., *Carex sylvatica* Huds., *Dryopteris carthusiana* (Vill.) H.P. Fuchs, *Dryopteris dilatata* (Hoffmann) A. Gray, *Dryopteris filix-mas* (L.) Schott, *Hyacinthoides non-scripta*, *Lonicera periclymenum*, *Luzula pilosa* (L.) Willd., *Milium effusum*, *Poa nemoralis* L., *Pteridium aquilinum* (L.) Kuhn, *Pulmonaria officinalis* L., *Stellaria holostea*, *Stellaria nemorum* L., *Veronica montana* L.

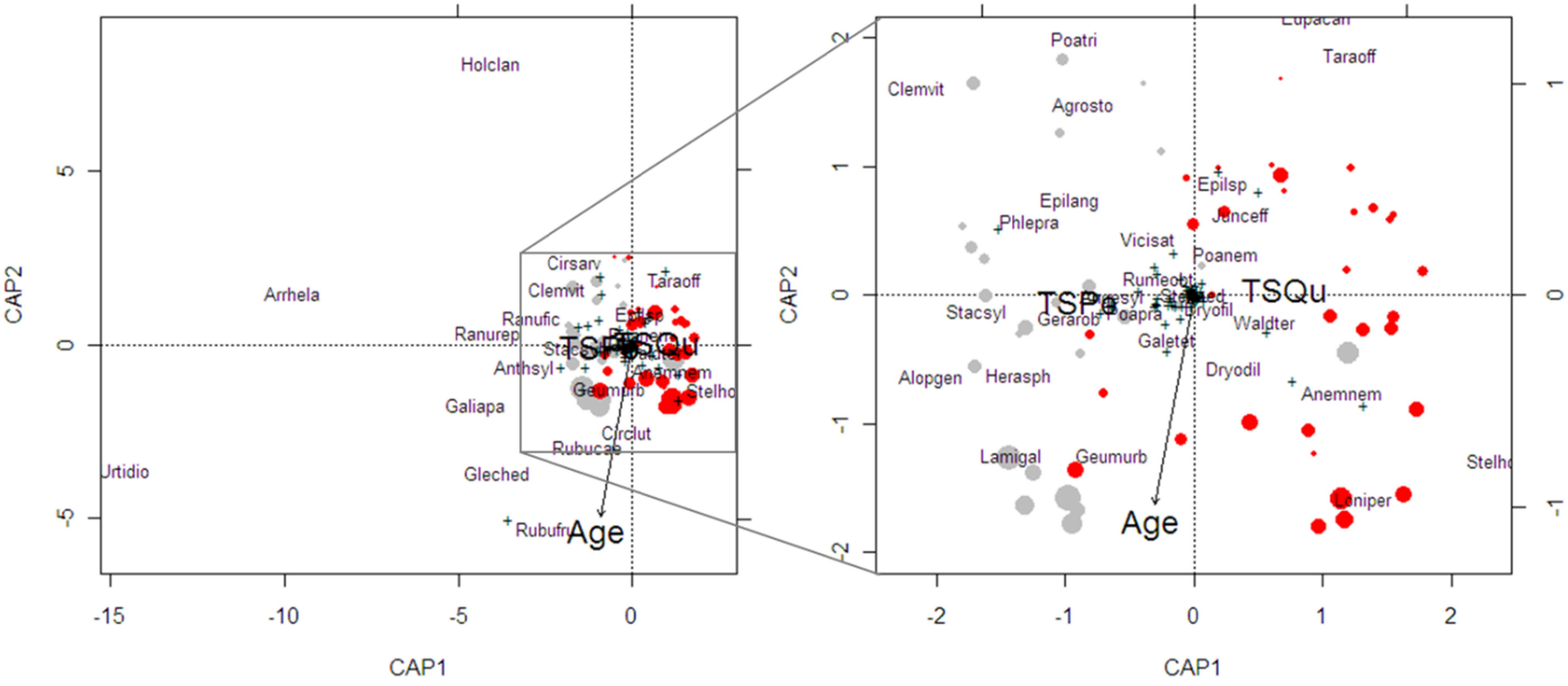

Figure 2.3 Triplot based on the PERMANOVA model of the herb layer with tree species and stand age as explanatory variables. Circles represent the positions of vegetation plots with grey circles: *Populus* plots and red circles: *Quercus* plots; Size of circles represents stand age; Species are either represented by + or their abbreviated names (Appendix A); TSPo: *Populus* plots; TSQu: *Quercus* plots.

Table 2.4 Herb layer species associated with a tree species (Phi association)

Species	TS	p
Anthriscus sylvestris (L.) Hoffmann	*Populus*	.001***
Arrhenatherum elatius	*Populus*	.001***
Epipactis helleborine	*Populus*	.001***
Urtica dioica	*Populus*	.002**
Dactylis glomerata L.	*Populus*	.003**
Galium aparine L.	*Populus*	.003**
Ranunculus repens L.	*Populus*	.006**
Heracleum sphondylium L.	*Populus*	.012*

TS: Tree species; *: $p < 0.05$; **: $p < 0.01$ and ***: $p < 0.001$

The total cover of the understory was significantly lower under *Quercus*. This is explained by pH-H_2O, but unexpectedly, not by cover of the overstory. The Ellenberg R value (Figure 2.4b), acid intolerant forest species (Figure 2.4e) and acid tolerant forest species (Figure 2.4f) are affected by the tree species and are explained by pH-H_2O. The Ellenberg N value is lower in *Quercus* than in *Populus* stands, N value is strongly influenced by Kj-N and to a lesser extent by pH-H_2O. The cover of light demanding forest species was significantly higher in *Populus* plots than in *Quercus* plots. Shade tolerant forest species, forest species and AFS are explained by stand age and isolation and are not affected by TS. Similar results were found for the number of forest species and the different subgroups at stand level (only results for cover at plot level shown) compared to their cover. Ellenberg value for light and S-herb are explained by the isolation from ancient forest (Figure 2.4g and h). C-herb is unaffected by TS, stand age or isolation but increases with increasing pH-H_2O.

When explaining the vegetation characteristics based on the environmental variables, most vegetation characteristics are explained by soil pH-H_2O (Table 2.2d). Understory cover, mN, mR, cover of light demanding and acid intolerant forest species decrease with pH-H2O, while acid tolerant forest species increase. For bio-P a positive relation is found with mL and a negative with shade tolerant forest species, light demanding forest species and acid intolerant forest species. In some cases N or C content further explained some vegetation characteristics.

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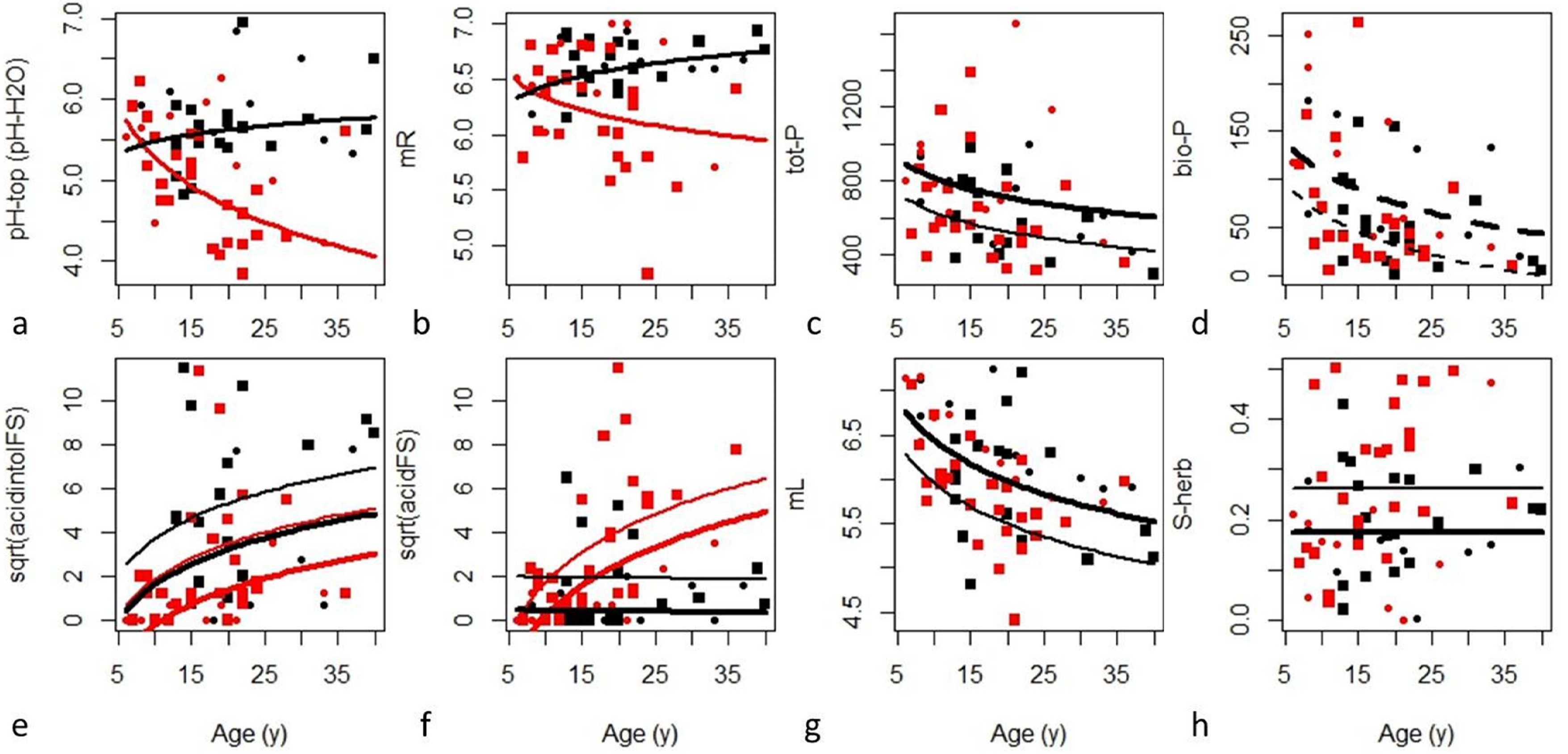


Figure 2.4 Graphs with linear mixed models for vegetation and environmental characteristics (a pH-H_2O; b mR; c tot-P; d bio-P; e acidintolFS; f acidFS; g mL; h S-herb, see Table 2.2). Black circles: Isolated *Populus* plots; Red circles: Isolated *Quercus* plots; Black squares: Adjacent *Populus* plots; Red squares: Adjacent *Quercus* plots; Black line: Model prediction for *Populus* (a,b,e,f); Red line: Model prediction for *Quercus* (a,b,e,f); Bold line: Model prediction for Isolated stands (c,e,f,g,h); Thin line: Model prediction for adjacent stands (c,e,f,g,h); Thin dotted line: Model prediction for sand fraction of 20% (d) and bold dotted line: Model prediction for sand fraction of 70% (d).

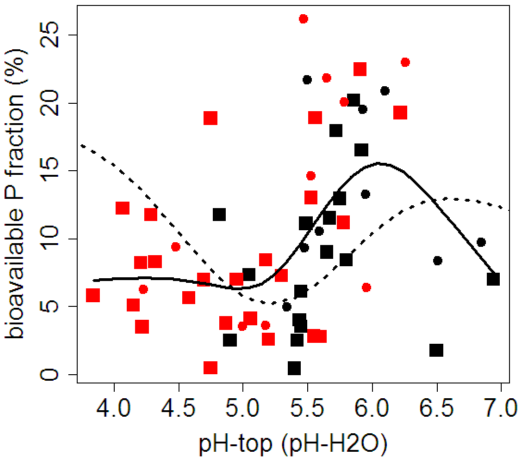


Figure 2.5 Graph with GAM model for bio-available P fraction (full line) with Forest as random effect and theoretical P availability (dotted line) based on Stevenson and Cole (1999). Black circles: Isolated *Populus* plots; Red circles: Isolated *Quercus* plots; Black squares: Adjacent *Populus* plots and Red squares: Adjacent *Quercus* plots.

2.4 Discussion

Our results indicate that the two contrasting tree species of our study (*Quercus* vs. *Populus*) are the drivers behind divergently developing forest ecosystems. One of the most striking differences between both tree species is the fast and strong acidification under *Quercus*, which contrasts with the stable situation under *Populus*. Also Ritter et al. (2003) found a fast acidification under *Q. robur* and *P. abies* and De Schrijver et al. (2012a) found lower pH in *Q. robur* stands compered to several other tree species. After only 20 to 30 yr of afforestation, the pH-H_2O of our *Quercus* stands reaches the Al buffer range with similar pH-H_2O levels as in ancient *Q. robur* and *F. sylvatica* forests on these soils (e.g. Baeten et al. 2009a). Available Al is toxic for many soil fauna and plant species (Falkengren-Grerup et al. 1995a; Tyler et al. 2002; Watanabe and Osaki 2002) and is associated with litter accumulation (e.g. Geissen and Brummer 1999). Litter accumulation may limit the colonisation of some forest plant species (Sydes and Grime 1981a; Eriksson 1995) whereas others might benefit from a moderate litter accumulation (Sydes and Grime 1981a). Including the litter thickness in the study might have further explained some of the tree species effects on the vegetation. To safeguard plant biodiversity from acidification in ancient forest, several studies advice to use post-agricultural forest as refugee zones for acid intolerant forest species (Dzwonko and Loster 1997; Bossuyt et al. 1999b; van Oijen et al. 2005; Valtinat et al. 2008). Based on the present study, it can be stated that for acidification sensitive soils this is only possible if the near neutral pH-H_2O of the former farmland is maintained by selecting moderately acidifying tree species.

The total soil P amount is a legacy of the historic fertilisation and is lower in older stands than in recent ones, most likely due to the longer period of fertilisation and the increasing intensity of fertilisation on farmland during the last decades (De Keersmaeker et al. 2004; De Schrijver et al. 2012b). The total P amount is not influenced by the tree species, but the available P fraction depends, among others, on soil pH-H_2O and thus indirectly depends on tree species. Our results more or less follow the theoretical model of Stevenson and Cole (1999) (Figure 2.5), but contrary to the theoretical model, P-availability remained low below a soil pH-H_2O of 5. It is, however, questionable whether minimising the bio-availability of P by trying to target a pH-H_2O of approximately 5 or lower will be sufficient to reduce competitors in post-agricultural forest with a large P stock.

Soil C and N content increased with stand age due to the accumulation of organic material. This finding is in agreement with Hooker and Compton (2003) and Zhang et al. (2010), but Ritter et al. (2003) found no accumulation of N. The soil C/N ratio was higher under *Quercus* in agreement with the moderate litter quality of *Q. robur* (Reich et al. 2005; Hobbie et al. 2006), but an effect of stand age seemed not significant in contrast with a clear age effect on pH. Ritter et al. (2003) shows a slightly lower C/N for *Q. robur* than for *P. abies* and a small increase with stand age only in the upper 5 cm of mineral soil. In some cases N or C content explained vegetation characteristics. However, I assume that this is most likely a proxy for age (e.g. a colonisation effect) rather than a specific effect of C or N.

The composition of the vegetation depends on the tree species, the isolation and age of the stand. *Quercus* stands cast more shade and have a progressively acidifying soil with a thicker litter layer, explaining the low total cover of the understory, compared to the *Populus* stands. In contrast, competitors and rough *Arrhenatherion*-grassland species grow vigorous and are associated with the fairly open canopy of the moderately acidifying *Populus*. The amount of light received by the understory fails to explain the understory cover in our setup despite the fact that two different measurement techniques were used (spherical densiometer, results not shown, and cover of trees and shrubs). This contrasts with the general assumption that shade casting is one of the most important overstory-understory effects (Barbier et al. 2008). The explanation might be found in the differences in light transmittance of *Populus* versus *Quercus* leaves, which are not taken into account in the measurements and by the fact that static, snapshot data were used which may not sufficiently reflect the variability in light transmittance over the growing season.

No clear tree species effects could be detected on the number (results not shown) or cover of colonised forest species and AFS. A significant difference possibly remains overseen by the limited number of older stands adjacent to ancient forest included in our study, as especially in these stands higher numbers of forest species were observed in contrast to the young and isolated post-agricultural forests. Nevertheless it is clear that both tree species create favourable conditions for forest herbs with different ecological amplitudes (cf. Dzwonko and Loster 1997; Aubert et al. 2004; van Oijen et al. 2005; Kooijman and Cammeraat 2010). I assume that acid tolerant species hampered by competitors in the *Populus* stands, due to a combination of high light availability, high nutrient content and near neutral soils. Acid intolerant species are influenced by Al toxicity in acid soils (Watanabe and Osaki 2002), which most likely explains their lower occurrence in *Quercus* stands. The indifferent reaction of shade tolerant species might be due to the fact that these small perennials notice little difference in light availability between a dense cover of a tree species and that of high grasses and herbaceous competitors. Furthermore, many shade tolerant species escape the shade of trees and competitors by their early growth and flowering optima. It can be surmised that overstory cover and soil acidification are constraining tree species effects for herb species recruitment. Acidifying tree species may have irreversible effects in the long run, permanently excluding establishment of acid intolerant species. *Populus* and *Quercus*, being the most frequently used trees in afforestations within the study region, were selected because of their contrasting ecology. Since many AFS are acid intolerant and specialised in light limited conditions (Hermy et al. 1999; De Keersmaeker et al. 2013) neither homogenous *Quercus* and *Populus* stands may be ideal for understory restoration on loamy soils.

The isolation of afforestations is known to hamper colonisation of AFS (Honnay et al. 1998; Bossuyt et al. 1999a; Verheyen and Hermy 2004). In our study the groups of shade and stress tolerant forest species were not very successful in colonising the isolated stands. This is explained by a colonisation credit, i.e. a lagging behind in shade and stress tolerant forest species which include many dispersal limited species (Hermy et al. 1999). This results in a limited herb layer cover in the shaded *Quercus* stands. Since vegetation development lagged behind, Ellenberg indicator value for light and CSR strategies are found to limp behind on the environmental change. Also Dzwonko (2001) mentioned earlier that Ellenberg indicator values fit less to the environmental conditions in post-agricultural forests than in ancient forests and Brunet et al. (2011) found that forest specialist plant species are effected by a gradient in fragmentation, while generalists were not affected. I deduce that understory recovery in isolated forests fails to fill in certain forest-typical ecological niches.

The *Populus* and *Quercus* stands in the presented work have been managed differently: poplars are planted as poles with wide spacing in between, while oaks are planted in high densities and thinned gradually. This tree species-specific management will most likely have influenced the results. The stem density of poplars is much lower resulting in a more scattered overstory and a slower increase towards canopy closure. In contrast canopy closure in *Quercus* stands is reached within a few years, remaining very dark for several years upon then, with only minor fluctuations caused by thinning. If the oaks were planted at wider spacing similar to the poplars, canopy cover will be lower which presumably will lead to a higher understory cover and cover of competitors. Furthermore, it would also reduce the initial litter fall and improve litter perturbation and decomposition (cf. Hobbie et al. 2006). Therefore, the results may not be interpreted as pure tree species effects but a combination of tree species effects and their typical management.

Our study was limited to 1st generation stands allowing an equal comparison between the tree species. However, poplars are cut after 20 to 40 yr depending on the cultivar in regular managed forest which strongly contrast the more than 100 yr cycles for *Quercus*. When no special attention is given to the understory, the clear cutting of poplars can have devastating effects on the vegetation development. However, if the cutting and removal of the trees is performed carefully, it is possible to even facilitate the colonisation of forest plant species by the short light exposure (De Keersmaeker et al. 2011b).

In our study land use before conversion to forest (crop field versus grassland) had little influence on the soil and vegetation of the post-agricultural forest. This is most likely explained by the intensive agricultural use of both land use categories and the shift from crop field to grassland. Most of the study sites on grassland (81%) were converted from crop field to grassland a few years prior to afforestation. In regions with clear differences in site conditions between crop fields and grasslands are present, results might differ between these land use categories.

In ten stands the accumulated cover of AFS was higher than 50%, equally occurring under *Populus* and *Quercus*. All of these *Populus* stands are rich in (ancient) forest species while in the *Quercus* stands only one or a few AFS, like *M. effusum* or *L. galeobdolon*, are present. I conclude that habitat restoration in post-agricultural forests is possible, if sites are selected adjacent to seed sources and P stocks are low.

From this study, I conclude that there is a strong effect of tree species and their typical management on soil and herb layer development. Therefore, it is of major importance that goals for soil development and understory restoration are taken into account when tree species are selected for afforestation projects. If a species rich herb layer with acid intolerant species is aimed at, selecting a tree species with good decomposable litter is a necessity. To suppress competitors a shady overstory (for example *Tilia* spp. or *C. betulus*) or a combination of *Populus* or *F. excelsior* with a dense shrub layer (cf. De Keersmaeker et al. 2004) should be incorporated. The shrub layer can generate shade but can also improve the litter quality. Furthermore, soil acidifying species like *Quercus* spp. or *F. sylvatica* might still be suitable to enlarge forest habitats that mainly contain acid and shade tolerant species. Because of their acidifying character and shady overstory, competitors are often completely absent under these tree species allowing shade and acid tolerant species like *H. non-scripta* to colonise more easily. By admixing trees or shrubs with good decomposable litter (e.g. *Tilia* spp., *C. betulus*, *Acer* spp., *F. excelsior* and *C. avellana*) within *Quercus* stands the litter quality and soil pH can be improved. Finally on sites where no colonisation of typical forest species might be expected, it is advisable to set ecosystem functioning as a prior goal rather than forest species colonisation (Aerts and Honnay 2011). In this case a more open overstory with dominance of competitors and grassland species underneath can be preferred above a species poor and very sparse understory, because the former is more likely to be more resilient and has a better ecosystem functioning (Tilman 1999), for example as a food source and shelter for fauna. Also at landscape level, biodiversity will benefit from heterogeneity in the selected tree species and management (Hartley 2002; Aerts and Honnay 2011). A better mechanistic understanding of tree species effects on ecosystem functioning and biodiversity in post-agricultural forests and a more complete overview on the effects of other tree species is, however, needed to optimally use tree species effects as a steering mechanism (Aerts and Honnay 2011).

3

Long term tree species effects on introduced forest herbs in a common garden

After: Thomaes, A., De Keersmaeker, L., Verschelde, P., Vandekerkhove, K., & Verheyen, K. Tree species determine the colonisation success of forest herbs in post-agricultural forests: Results from a 9 yr introduction experiment. Biological conservation, DOI 10.1016/j.biocon.2013.10.024

Abstract

The limited dispersal and environmental limitation of AFS hampers the restoration of herb layer communities in post-agricultural forests. Environmental limitation is often caused by the high availability of soil nutrients, in particular P, which can aggravate competitive exclusion of AFS by nutrient-demanding species. I monitored the survival, growth and reproduction of six AFS with variable ecological amplitudes for light and soil acidity according to expert-based indicator values. These AFS were introduced under eight tree species that have created specific levels of light and soil acidity within 30 years after plantation on farmland. Acidifying tree species severely limited the survival of all introduced AFS except *H. non-scripta*. I observed the highest growth of AFS in stands with a low light level. Temporary canopy gaps promoted the survival of AFS, but also provoked dominance by light-demanding competitors before canopy closed again. The two hemicryptophyte AFS, *P. elatior* and *Oxalis acetosella* L., increased by means of sexual reproduction, while the geophytes (*A. nemorosa, M. perennis, P. aquilinum,* and *H. non-scripta*), mainly expanded vegetative. As a conclusion, I advise managers that aim to restore a species-rich forest herb layer in post-agricultural forest, not to promote strongly acidifying tree species. The regulation of the level and/or frequency of stress, soil acidity and shade in this case, can be used as a management tool to mitigate the competitive exclusion promoted by P eutrophication.

3.1 Introduction

Plant distribution patterns are structured both by dispersal and environmental limitation (Eriksson and Ehrlén 1992; Pulliam 2000; Hermy and Verheyen 2007; Baeten et al. 2009b). Consequently, restoration ecology needs to solve both the propagule dispersal barriers as well as the habitat unsuitability to be successful (e.g. Martin and Wilsey 2006; Standish et al. 2007). Solving the dispersal obstruction infer spreading habitat restoration efforts in time and space gradually outwards from the propagule sources (e.g. Hermy et al. 1999; Herault et al. 2005). In some cases, introductions can be considered. However, a review of 249 plant introductions indicated that large numbers of plants are needed, that only 52% of the reintroductions were successful in terms of survival, and only 16% of the studies reported successful fruiting (Godefroid et al. 2011).

While dispersal limitation can be solved, it may be far more difficult to determine the habitat unsuitability, causing many plant introductions to fail (Ehrlén and Eriksson 2000; Bottin et al. 2007; Godefroid et al. 2011). Therefore, one of the prime objectives of habitat restoration is a proper identification of suitable habitat patches (Tischew and Kirmer 2007; Menges 2008). Menges (2008) related habitat unsuitability with exclusion of target species by competitors (i.e. competitive exclusion) and associated the altered competition with habitat modifications and unsuitable levels of disturbance or stress. Site preparation (e.g. litter removal) is one of the solutions suggested to increase success of plant reintroductions (Menges 2008; Godefroid et al. 2011). However, habitat suitability must be seen in the light of all life stages, including adult growth, flowering, fruiting, seed germination and seedling recruitment, as ontogenetic shifts in habitat requirements regularly occur (Drayton and Primack 2012).

AFS have been found to be strongly limited by both propagule dispersal and environmental limitation (Ehrlén and Eriksson 2000; Verheyen and Hermy 2001b; Verheyen et al. 2003a; Baeten et al. 2009b). The behaviour of AFS in post-agricultural forests is still poorly understood, e.g. indicator values by Ellenberg et al. (1992) or Hill et al. (1999) are based on expert judgment of species performance in typical forest vegetation, i.e. in long-established forests. A recent study indicated that also among species listed as slow colonizers (AFS), colonisation rates in post-agricultural forest showed a high variability (Brunet et al. 2012). To study the effects of habitat suitability of post-agricultural forests on performance of colonizing AFS, a long-term monitoring of all life stages is required. Some species, like many AFS, reproduce mainly clonally and not or infrequently by seed (Holderegger et al. 1998; den Ouden 2000; Baeten et al. 2009c). Instead the change in total cover would be a better overall success factor as it depends on survival, vegetative and generative expansion.

The soil in many sites considered for restoration is chemically enriched due to historic fertilisation. P enrichment in particular has been found to be a key factor limiting biodiversity restoration (e.g. Noe et al. 2001; Wassen et al. 2005). Consequently, competition between target species and competitors is outbalanced (Menges 2008). For example, performance of stress-tolerant species and habitat specialists in restored grassland was affected by residual fertility (Pywell et al. 2003; Smith et al. 2003). The restoration of AFS in forests established on former agricultural land (i.e. post-agricultural forest) is hampered by the altered competition between AFS and P-opportunistic species, like *U. dioica* or *G. urbanum* (Baeten et al. 2009b). Removal of soil P stocks, e.g. by topsoil removal, is often not feasible, nor desirable (Torok et al. 2011). Therefore, establishing stress (or disturbance) factors tolerated by the target species but not by the competitors might be far more effective for restoration ecology on P enriched soils. For example, De Keersmaeker et al. (2004) found that increasing shade in post-agricultural forests counterbalanced the P-altered relation between competitors and AFS.

I studied the colonisation success of AFS that were introduced in post-agricultural forest. Tree species are considered as ecosystem engineers with a strong impact on the environmental conditions and resource availability for forest understories (Jones et al. 1997). Light availability and soil acidification level are identified as main factors explaining this influence (Barbier et al. 2008). The main goal of our study was to determine which tree species created the optimal environmental conditions (shade, soil acidification, litter quantity) in post-agricultural forest for colonisation by AFS. I assume a trade-off between the decrease of the survival rate of AFS, controlled by soil acidification, and the decrease of competitive exclusion, controlled by canopy closure and soil acidification.

The previous chapter focused on the spontaneous colonisation in a chronosequence of afforestations. Here, I will study more tree species but the age between tree species is fixed. Long term in situ introduction experiments can reveal the overall tree species effect, including the effect of litter and indirect effects by altered competition between herb layer species, on the different life stages of AFS in post-agricultural forest (Dzwonko and Loster 1997). For this purpose, I have set up an introduction experiment that comprised six AFS introduced in ten stands of eight tree species. The introduction was used to guarantee equal comparison between species as spontaneous colonisation happens infrequently and strongly depends on propagule dispersal (chapter 2). Consequently the main question will be whether tree species can influence the survival and spread of initially established individuals. The study site can be considered as a common garden and the selected tree species cover a full factorial set up of explanatory factors, i.e. light level and soil acidification level. Likewise, AFS were selected based on their contrasting ecological optimum for shade and soil acidity according to expert-based indicator values. I hypothesized that (1) Species that impose a high level of shade will reduce competitive exclusion of AFS and thus promote the introduction success but the effect might depend on shade tolerance and Raunkiear life form of the introduced AFS. (2) Tree species that cause soil acidification can limit colonisation by acid intolerant AFS but can promote acid tolerant AFS by reducing nutrient-demanding competitors.

3.2 Material & Methods

3.2.1 Research site and plant introduction

Mortagne forest was selected as research site. Ten stands are selected and grouped in ecological groups (Table 3.3) according to their contrasting tree species effects on soil acidification (ACIDITY) and shade casting (SHADE) based on previous research (see chapter 1). For soil acidification, tree species can be grouped in two rather separated groups, soil-acidifying species that quickly reach the Aluminium (Al) buffer range and species that only moderately acidify the soil when planted on well limed agricultural land (van Oijen et al. 2005; De Schrijver et al. 2012a; chapter 2). The design included two stands of each combination of strongly acid/moderately acid and strongly/moderately shaded. Two more stands were added, a clearcut area and a strongly shaded stand that was cut 5 yr after the introduction (Table 3.1). The tree and shrub layer of the clearcut area were cut in the winter 2003-2004 prior to the introduction. The shrubs and poplar stumps resprouted and many *Salix* spp. seedlings recruited on the logging tracks. In the winter of 2007-2008 the shrub layer of the second poplar stand was coppiced and the following winter the poplars were cut. Two N2-fixing tree species are included in the experiment: *A. glutinosa* and *R. pseudoacacia*. The soil Nitrogen (N) concentration under *A. glutinosa* was high, which was not the case under *R. pseudoacacia* (Dossche 1998).

For this experiment six AFS (Table 3.2) with varying ecological tolerance for soil acidity and shade and absent in Mortagne forest were selected from the Flemish list of AFS (Table 3.3): *P. elatior*, *A. nemorosa*, *M. perennis*, *P. aquilinum*, *H. non-scripta* and *O. acetosella*. In the study region, *P. aquilinum* is one of the few AFS that is typical for acid soils (De Keersmaeker et al. 2013). As opposed to some regions in the UK where it also occurs on many open habitats, including moorlands (Peterken and Game 1984; Hermy et al. 1999), this fern species is rarely found outside forest in our study area

(Hermy et al. 1999). In March 2004, adults of these species were planted as the recruitment of seeds is known to be very low (also see chapter 4 and 5).

Like many AFS, four of the species are geophytes that reproduce infrequently by seeds and mainly expand clonally (e.g. Holderegger et al. 1998; den Ouden 2000). The two hemicryptophytes, *P. elatior* and *O. acetosella* reproduce mainly by seeds (Berg 2002). I expect that the colonisation of hemicryptophytes is consequently more sensitive for stress as they depend on vulnerable seedlings for recruitment. Furthermore, the hemicryptophytes and two geophytes (*M. perennis* and *P. aquilinum*) are summer-green, thus might respond differently to the light availability. Most of the species were retrieved from a commercial nursery specialised in wild plants while *O. acetosella* and *M. perennis* were collected in the Aelmoeseneie forest (50.98° N, 3.88° E), an ancient forest on a sandy loam soil. The original soil was washed off the roots to introduce the plants bare rooted. Plants were weighed before planting. One individual of each of the six species were planted in a circle (radius 30 cm, 30 cm between species) around 19 or 20 grid points of a 10 x 10 m grid in each stand, 199 points in total (Table 3.1, Figure 3.1). *P. elatior* and *H. non-scripta*, resulted from seedlings, others came from vegetative reproduction. The *P. elatior* retrieved from the nursery, were clusters of up to about 10 plants planted together in a flowerpot. These clusters were planted as a single replicate on each grid point. In the clearcut stand, some grid points were shifted to avoid positioning them on the logging tracks. Plots remained unmarked and unfenced and no site preparation was performed since I wanted to mimic a natural colonisation as much as possible.

3.2.2 Measurements

During the first 3 yr, the plants were visited 3 times a year (end of March, mid-May and mid-July) and a set of 6 variables was measured (Thomaes et al. 2007). Since then, the plants were visited yearly in mid-May and a reduced set of variables was measured (Table 3.2) based on the evaluation of the earlier data (Thomaes et al. 2007). The cover was estimated in classes by comparing plants with reference areas of cover classes indicated on a cardboard, cover of larger plants was estimated in classes of 0.5 m². Furthermore, the gender of the dioecious *M. perennis* was determined because of differences in clonal growth and shade tolerance between genders (Wade 1981; Wade et al. 1981). In most cases both flowering forms of heterostylous *P. elatior* occurred within the introduced cluster of plants at each grid point, hence they were not distinguished. One grid point was removed from the results (198 remaining) since a tree fell on it and eliminated all except one of the introduced species.

Table 3.1 Description of the selected stands according to their ecological groups (soil acidifying (ACIDITY) and shade casting (SHADE)), with number of grid points for plant introduction and thinning performed in the winters of 2007-2008 and 2011-2012

Stand	Tree species	ACIDITY	SHADE	Nr of grid points	2007-08	2011-12
Alnus5	*Alnus glutinosa*	ACID	MOSH	20	X	X
Robinia5	*Robinia pseudoacacia*	ACID	MOSH	20	0	0
Fagus6	*Fagus sylvatica*	ACID	STSH	20	0	X
Quercus6	*Quercus rubra*	ACID	STSH	20	X	0
Fraxinus1	*Fraxinus americana*	MOAC	MOSH	20	X	X
Fraxinus3	*Fraxinus americana*	MOAC	MOSH	19	X	X
Acer8	*Acer pseudoplatanus*	MOAC	STSH	20	0	X
Tilia4	*Tilia cordata*	MOAC	STSH	20	X	X
Populus8	*Populus* x *canadensis* Robusta’	MOAC	STSH	20	XX	0
Clearcut6	Clearcut of *Populus* x *canadensis* ‘Harff’	MOAC	CLEAR	20	0	0

ACID: Acidifying tree species, MOAC: moderately acidifying tree species, MOSH: moderately shaded, STSH: strongly shaded, CLEAR: clearcut, X: thinned, XX: clearcut 2008-2009 and 0: not thinned.

Table 3.2 General information on introduced plants: Plant origin, initial plant weight (mean ± s.d.), life forms (L, He: hemicryptophytes and Ge: geophytes), acid and shade tolerance (Table 3.3) and measured performance indicators of each plant species (PlC: Plant cover, Np: Number of pedicels; Ns: Number of seedlings)

Ancient forest species	Plant origin	Initial plant weight (g)	L	Acid tolerance	Shade tolerance	PlC	Np	Ns
P. elatior	nursery	102 ± 86	He	Intolerant	Intermediate	X	X	X
A. nemorosa	nursery	6.6 ± 3.6	Ge	Intermediate	Intermediate	X	X: flowers	X
M. perennis	Aelmoeseneie f 50.98°N, 3.88°E	6.5 ± 3.5	Ge	Intolerant	Tolerant	X	-	X
P. aquilinum	nursery	68 ± 62	Ge	Tolerant	Intolerant	X	-	X
H. non-scripta	nursery	21 ± 8	Ge	Intermediate	Intermediate	X	X	X
O. acetosella	Aelmoeseneie f	2.1 ± 1.1	He	Tolerant	Tolerant	X	X: flowers	X

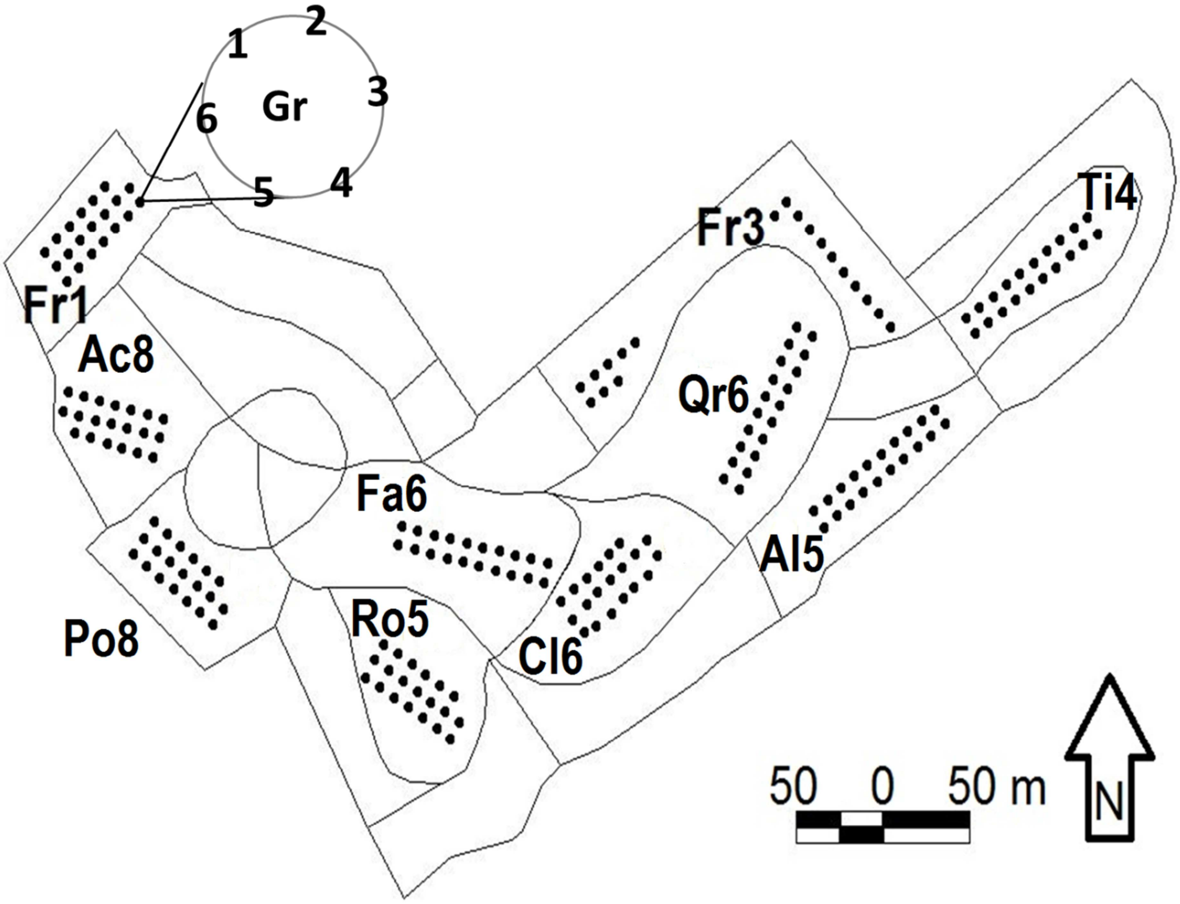
X: measured for this species and -: not measured for this species.

Table 3.3 Frequently occurring AFS on loam and sand loam soils from the list of Flemish AFS (De Keersmaeker et al. 2011a) with their mean and 75% range of occurrence for Hill mR and mL values (Hill et al. 1999) for 6473 forest plots in Flanders (Cornelis et al. 2009), R and L values from Hill et al. (1999) and Ellenberg et al. (1992). Species in bold are selected species for introduction.

Species	mR		R		mL		L	
	Mean	Range	Hill	Ellenb	Mean	Range	Hill	Ellenb
Acid tolerant species (mR range < 5.0) ordered by decreasing shade tolerance								
Milium effusum	5.5	4.7-6.5	6	5	5.0	4.5-5.6	4	4
Oxalis acetosella	**5.3**	**4.6-6.1**	**4**	**4**	**5.0**	**4.5-5.6**	**4**	**1**
Maianthemum bifolium	4.5	3.1-5.8	3	3	5.3	4.7-5.9	3	3
Convallaria majalis L.	4.9	3.7-5.9	6	*	5.3	4.8-5.8	5	5
Athyrium filix-femina	5.6	4.8-6.5	5	*	5.4	4.7-6.2	5	3
Lonicera periclymenum	4.9	3.3-6.2	5	3	5.6	4.9-6.2	5	6
Holcus mollis	4.8	3.4-5.9	3	2	5.7	5.0-6.3	6	5
Pteridium aquilinum	**4.2**	**3.0-5.5**	**3**	**3**	**5.7**	**5.1-6.1**	**6**	**6**
Carex pilulifera L.	4.1	3.0-5.3	3	3	5.9	5.3-6.4	7	5
Intermediate acid tolerant species (mR range < 5.4) ordered by decreasing shade tolerance								
Lamium galeobdolon	6.0	5.3-6.7	7	7	5.0	4.4-5.7	4	3
Hedera helix	5.9	5.0-6.8	7	*	5.2	4.5-5.9	4	4
Anemone nemorosa	**5.8**	**5.1-6.5**	**5**	*****	**5.1**	**4.6-5.7**	**5**	*****
Polygonatum multiflorum	5.8	5.0-6.7	7	6	5.2	4.6-5.8	4	2
Hyacinthoides non-scripta	**5.6**	**5.0-6.3**	**5**	**7**	**5.1**	**4.7-5.7**	**5**	**5**
Stellaria holostea	5.8	5.1-6.5	6	6	5.2	4.8-5.8	5	5
Carex remota	5.8	5.0-6.6	6	*	5.5	4.8-6.2	4	3
Acid intolerant species (mR range ≥ 5.4) ordered by decreasing shade tolerance								
Mercurialis perennis	**6.4**	**5.8-6.9**	**7**	**8**	**4.6**	**3.8-5.6**	**3**	**2**
Allium ursinum	6.5	6.0-6.8	7	7	4.6	4.0-5.2	4	2
Vinca minor	6.1	5.4-6.8	7	7	4.8	4.2-5.4	4	4
Arum maculatum	6.4	5.8-6.8	7	7	5.1	4.3-5.7	4	3
Paris quadrifolia	6.2	5.6-6.7	7	7	5.1	4.4-5.6	3	3
Primula elatior	**6.3**	**5.8-6.8**	**7**	**7**	**5.2**	**4.5-5.9**	**4**	**6**
Circaea lutetiana	6.3	5.7-6.8	7	7	5.3	4.5-5.9	4	4
Carex sylvatica	6.1	5.5-6.7	6	6	5.2	4.6-5.8	4	2
Viola riviniana Reichenb. /*reichenbachiana* Jord. ex Boreau	6.0	5.4-6.7	5/7	4/7	5.2	4.6-5.8	6/4	5/4

*: Species with broad amplitude

Figure 3.1 Grid points of the introduction of ancient forest species within Mortagne forest. Abbreviated stand names: Ac8: Acer8, Al5: Alnus5, Cl6: Clearcut6, Fa6: Fagus6, Fr1: Fraxinus1, Fr3: Fraxinus3, Po8: Populus8, Ro5: Robinia5, Ti4: Tilia4 and Qr6: Quercus6 (Table 3.1). Gr: grid point, 1: position of *Primula elatior*, 2: *Hyacinthoides non-scripta*, 3: *Anemone nemorosa*, 4: *Pteridium aquilinum*, 5: *Oxalis acetosella* and 6: *Mercurialis perennis.*



Within 1 m of each grid point, the species and cover (Londo scale) of the herb layer was registered in May and July 2004 and the highest cover was used. Light measurements were performed mid-July 2004 and early April, mid-May and mid-July 2005, 20 cm above the ground at each grid point and on nearby open fields with an LAI-2000 Plant Canopy Analyzer. Shade casting was calculated as the percentage of light reduction at a grid point as compared to the open field. Mineral soil samples at a depth of 0-10 cm were collected in July 2005 as a mixture of four subsamples gathered 1 m away from each grid point. Soil samples were analysed on bio-available P (bio-P, Bray and Kurtz 1945), Kjeldahl N (heat destruction with H_2SO_4 and K_2SO_4/$CuSO_4$ catalyst, analysed by titration in H_3BO_3), pH-KCl and pH-H_2O (glass electrode). Litter was collected in the beginning of February 2004 within a circle with 19 cm radius near each grid point, dried (at 45 °C during 1 month) and weighted.

3.2.3 Statistics

The stands were characterised for soil acidity (pH-KCl and pH-H_2O) and litter accumulation (dry weight), degree of shade casting (light measurements and sum of the herb layer cover) and soil fertility (bio-P and Kjeldahl N) in order to evaluate the a priori grouping of the stands with ANOVA and Tukey multiple comparisons. The sum of the herb layer cover was a better estimate for the degree of shade than the Ellenberg value for light since little shade tolerant species were present in the forest.

To evaluate the success of the introductions three subsequent analyses were performed to see how tree species effects influenced (1) the survival, (2) the cover and (3) generative reproduction of the introduced plants. Finally, the change in total cover of the introduced species at stand level was compared as an overall success rate.

Firstly, survival of species was examined by Kaplan-Meier survival analysis. Five tests were applied to answer whether there is a difference (1) between stands, (2) between acidifying and moderately acidifying tree species, between strongly and moderately shade casting trees within (3) acidifying and (4) moderately acidifying trees and (5) between clearcut and moderately shade casting within moderately acidifying trees. A Bonferroni correction was applied for the five tests.

Secondly, generalised additive mixed models (GAMM) were built explaining plant cover by soil acidification, shade casting and year (representing the age of the introduced plants). Initial plant weight was used as confounding variable and a smoother (initial k=4) was applied on year. Logarithmic transformation was applied on initial plant weight and plant cover. The individual plant, nested within stand, was used as random intercept to account for random variation between individual plants and stands. Backwards selection was applied on the variables, their interaction and the smoother using a p-value of 1% on the maximum likelihood test. The structure of the model is:

Log (Cover) ~ 1 + s(Year) * SHADE * ACIDITY + log(Plant weight) + (1|stand) + (1|plant)

Thirdly, success rates in terms of the generative reproduction: flowering (in any of the 9 yr), producing seedlings (in any of the 9 yr) and recruitment into new adults (noted in 2011) were evaluated by means of generalised linear mixed models (GLMM). Tree species groups for acidification and shade casting were used as variables, stand was used as random intercept and backwards selection was applied by a p-value of 1% on the maximum likelihood test. The structure of the model is:

Success rate ~ 1 + SHADE * ACIDITY + (1|stand)

All statistics were performed in R 2.15.2 with the library's survival, lme4 and mgcv (Therneau and Grambsch 2000; R Development Core Team 2009; Wood 2011; Bates et al. 2012; Therneau 2013).

3.3 Results

The a-priori grouping of the stands according to the soil acidification level was in agreement with pH-H_20 and pH-KCl of the top soil and forest floor biomass measured in our experiment (Table 3.4). The pH-H_2O of the four strong acidifying tree species was below 4.2 which is the upper limit of the Al buffer range, meaning that sensitive species can suffer from Al-toxicity (Stevenson and Cole 1999; Fisher and Binkley 2000; Lukac and Godbold 2011). Furthermore, these tree species exhibit a much stronger build-up of the forest floor. Within the moderately acidifying tree species, there is a gradient from the *Acer* stand, with a pH-H_20 and pH-KCl not significantly higher than the *Fagus* stand, up to the *Populus* stand. The subdivision in strongly and moderately shaded stands was significantly at the level of the group but was only weekly present at the stand level. Only the clearcut and the *Robinia* stand differed clearly in shade casting from the other stands. Nevertheless, the stands of the two groups clearly differed in herb layer cover. Finally, some small differences were found for bio-P, one of the *Fraxinus* stands has the lowest bio-P while the *Acer* stand has the highest bio-P. Like in previous research (Dossche 1998), the stand of *Alnus* had strongly increased N levels while this was not the case for *Robinia*.

Table 3.4 Mean values (± s.e.) for each stand and ecological group of stands analysed with ANOVA and Tukey multiple comparison. Equal letters in superscript indicate values which are not significantly different

ACIDITY	stand	$pH\text{-}H_2O$		pH-KCl		Log(litter+1) ($g.m^{-2}$)	
	p	$< 2*10^{-16***}$	$< 2*10^{-16***}$	$< 2*10^{-16***}$	$< 2*10^{-16***}$	$< 2*10^{-16***}$	$< 2*10^{-16***}$
	df	1,197	9,189	1,197	9,189	1,197	9,189
ACID	Alnus	4.04 ± 0.02^{a}	3.82 ± 0.02^{a}	3.27 ± 0.01^{a}	3.09 ± 0.01^{a}	1973 ± 126^{a}	1694 ± 131^{bc}
	Robinia		4.05 ± 0.03^{b}		3.32 ± 0.02^{b}		2141 ± 276^{ab}
	Quercus		4.13 ± 0.04^{b}		3.34 ± 0.03^{b}		1136 ± 101^{cd}
	Fagus		4.16 ± 0.03^{bc}		3.33 ± 0.02^{b}		2922 ± 264^{a}
MOAC	Acer	4.89 ± 0.05^{b}	4.37 ± 0.04^{cd}	3.94 ± 0.04^{b}	3.46 ± 0.03^{bc}	465 ± 44^{b}	855 ± 93^{de}
	Tilia		4.54 ± 0.04^{de}		3.56 ± 0.03^{cd}		795 ± 127^{def}
	Fraxinus3		4.67 ± 0.04^{e}		3.75 ± 0.03^{d}		639 ± 87^{ef}
	Fraxinus1		5.01 ± 0.04^{f}		4.10 ± 0.07^{e}		513 ± 44^{f}
	Clearcut		5.08 ± 0.08^{f}		4.09 ± 0.08^{e}		0 ± 0^{f}
	Populus		5.65 ± 0.06^{g}		4.69 ± 0.07^{f}		0 ± 0^{f}

(table 3.4 continued)

SHADE	Stand	Log (∑herblayer)		Canopy April (%)		Canopy May (%)		Canopy July 04/05 (%)	
	p	$< 2*10^{-16***}$	$< 2*10^{-16***}$	$< 2*10^{-16***}$	$< 2*10^{-16***}$	$< 2*10^{-16***}$	$< 2*10^{-16***}$	$< 2*10^{-16***}$	$< 2*10^{-16***}$
	df	2,196	9,189	2,196	9,189	2,196	9,189	2,196	9,189
CLEAR	Clearcut	96.20±9.95^{a}	96.20±9.95^{a}	25.74±1.70^{a}	25.74±1.70^{a}	69.91±3.96^{a}	69.91±3.96^{a}	47.42±4.25/ 92.67±1.27^{a}	47.42±4.25/ 92.67±1.27^{a}
MOSH	Robinia	110.42±6.03^{a}	122.03±11.04^{a}	59.71±1.47^{b}	47.85±3.56^{b}	94.21±0.62^{b}	87.93±1.27^{b}	96.35±0.36/ 97.22±0.26^{b}	93.43±0.88/ 95.79±0.59^{b}
	Fraxinus1		120.38±14.96^{a}		62.44±1.39^{c}		97.48±0.41^{c}		97.21±0.53/ NA^{c}
	Alnus		108.55±12.02^{a}		67.22±2.40^{c}		95.43±1.12^{c}		98.11±0.35/ 99.07±0.18^{c}
	Fraxinus3		89.71±8.99^{a}		61.43±2.16^{c}		96.09±0.58^{c}		96.68±0.53/ 96.79±0.44^{c}
STSH	Acer	10.00±2.19^{b}	32.05±7.77^{b}	66.25±0.60^{c}	68.62±2.06^{c}	98.60±0.11^{c}	98.27±0.37^{c}	99.26±0.16/ 97.57±0.07^{c}	96.65±0.35/ 98.88±0.23^{c}
	Populus		9.43±3.53^{c}		66.67±1.02^{c}		98.06±0.16^{c}		98.97±0.08/ 99.23±0.09^{c}
	Quercus		6.55±4.04^{c}		69.91±1.08^{c}		98.22±0.25^{c}		97.35±0.40/ 99.20±0.09^{c}
	Tilia		1.65±0.17^{cd}		64.09±0.55^{c}		99.55±0.04^{c}		96.52±0.20/ 99.73±0.01^{c}
	Fagus		0.33±0.15^{d}		61.99±0.63^{c}		98.91±0.15^{c}		98.37±0.26/ NA^{c}

(table 3.4 continued)

stand	Bio-P (mg.kg^{-1})	stand	Log(Kj-N*100) (%)
p	$< 2*10^{-16***}$	p	$< 2*10^{-16***}$
df	9,189	df	9,189
Fraxinus3	23.29 ± 2.04^{a}	Fagus	$0.15 \pm 0.01\%^{a}$
Robinia	28.18 ± 3.16^{ab}	Tilia	$0.15 \pm 0.004\%^{ab}$
Tilia	28.71 ± 3.19^{ab}	Quercus	$0.17 \pm 0.004\%^{bc}$
Quercus	36.29 ± 4.28^{ab}	Clearcut	$0.20 \pm 0.01\%^{cd}$
Fraxinus1	39.07 ± 2.04^{ab}	Fraxinus3	$0.23 \pm 0.01\%^{de}$
Populus	44.20 ± 3.43^{bc}	Acer	$0.24 \pm 0.02\%^{de}$
Alnus	44.56 ± 6.54^{bc}	Populus	$0.25 \pm 0.02\%^{de}$
Fagus	47.21 ± 3.22^{bc}	Robinia	$0.26 \pm 0.01\%^{e}$
Clearcut	59.54 ± 3.72^{cd}	Fraxinus1	$0.27 \pm 0.01\%^{e}$
Acer	76.52 ± 7.17^{d}	Alnus	$0.48 \pm 0.02\%^{f}$

ACIDITY: Ecological groups of tree species according to their soil acidifying, SHADE: Ecological groups of tree species according to their shade casting, ACID: Acidifying tree species, MOAC: moderately acidifying tree species, MOSH: moderately shaded, STSH: strongly shaded, CLEAR: clearcut; ∑herblayer: Sum of the cover of the herb layer species; ***: p <0.001; NA: Not applied.

The survival of all introduced species differed between the stands except for *H. non-scripta* (Table 3.5). This species is listed as intermediate for acidity and shade, while this species seems to have broad amplitude and is resistant to acidification and shade. The other intermediate and intolerant species for acidity, *P. elatior, A. nemorosa* and *M. perennis,* all had low survival under the acidifying tree species (Figure 3.2). Survival of *M. perennis* (shade tolerant) was higher on the clearcut, than in the other moderately shaded stands. Survival of this species was low in stands with low light levels. In contrast, survival of *P. elatior* (intermediate for shade) was lower on the clearcut area than in the other stands. The shade intolerant *P. aquilinum* initially survived well at the clearcut but the number of plants fell back as the canopy closed again. In the other stands *P. aquilinum* disappeared nearly completely after one year. After the Poplars were cut in 2008, *P. aquilinum* did not reappear, showing that the root stocks most likely had died off. The shade-tolerant species *O. acetosella* strongly declined in number in all stands. In the strongly shaded stands only two individuals survived for more than 2 years, again near the border of the stand in more light rich conditions. In the moderately shaded stands only a few plants survived, while the numbers steadily declined on the clear-cut area until the last plant disappeared in 2010.

The cover of the introduced species was influenced by both the level of shading and soil acidification (Table 3.6, Figure 3.3). The cover of the acid intolerant *P. elatior* and intermediately tolerant *A. nemorosa* was lower in the strongly than in the moderately acidified stands. Growth of the third acid intolerant species (*M. perennis*) in strongly acidified stands was not evaluated as this species did not survive long. The cover of *P. elatior* seems to converge in the last years, but this is explained by the fact that only relatively large individuals survived in the acidified stands. In contrast, the cover of the acid tolerant species *O. acetosella* and the intermediately tolerant *H. non-scripta* was not affected by soil acidification.

The level of shading was important for the cover of all herbaceous species. The cover of the intermediate shade tolerant *H. non-scripta* and the shade tolerant *O. acetosella* was higher in the shaded stands than in the moderately shaded stands. The cover of *O. acetosella* on the clearcut initially increased rapidly, but declined when competitive herbs became dominant and finally disappeared in 2010. The shade tolerant *M. perennis* first performed less on the clearcut than elsewhere but when the canopy closed, plants on the clearcut became bigger than in the stands with moderately levels of shade. The intermediate shade tolerant *A. nemorosa* initially had a higher cover in the moderately shaded stands than in the strongly shaded stands, but the cover in both stand types converged and was equal at the end of the monitoring.

Table 3.5 Chi^2 and p-value of Kaplan-Meier analyses on the survival of introduced ancient forest species. Species sorted by acid tolerance and shade tolerance (Table 3.2)

	Stand		ACIDITY		MOSH vs. STSH within ACID		MOSH vs. STSH within MOAC		MOSH vs. CLEAR within MOAC	
N	20,19,20,20,20,20,19, 20,20,20		100,98		40,40		39,60		39,19	
	Chi^2	p	Chi^2	P	Chi^2	p	Chi^2	p	Chi^2	p
P. elatior	103.0	<0.0001***	91.3	<0.0001***	0.0	0.957NS	0.1	0.734NS	7.2	0.007*
A. nemorosa	38.1	<0.0001***	21.8	<0.0001***	2.6	0.106NS	0.6	0.431NS	0.0	0.943NS
M. perennis	163.0	<0.0001***	63.3	<0.0001***	2.9	0.089NS	13.4	0.0003**	2.3	0.132NS
P. aquilinum	80.0	<0.0001***	13.5	0.0002**	2.0	0.152NS	4.8	0.029NS	42.7	<0.0001***
H. non-scripta	19.7	0.020NS	1.6	0.202NS	0.5	0.473NS	2.5	0.110NS	0.0	0.993NS
O. acetosella	34.8	<0.0001***	0.5	0.459NS	12.5	<0.0001***	14.6	0.0001***	7.0	0.008*

ACIDITY: Ecological groups of tree species according to their soil acidifying, ACID: Acidifying tree species, MOAC: moderately acidifying tree species, MOSH: moderately shaded, STSH: strongly shaded, CLEAR: clearcut; Bonferroni correction: *: p <0.05/5 =0.01; $^{**:}$ p <0.01/5 =0.002; ***: p <0.001/5 =0.0002 and NS: not significant

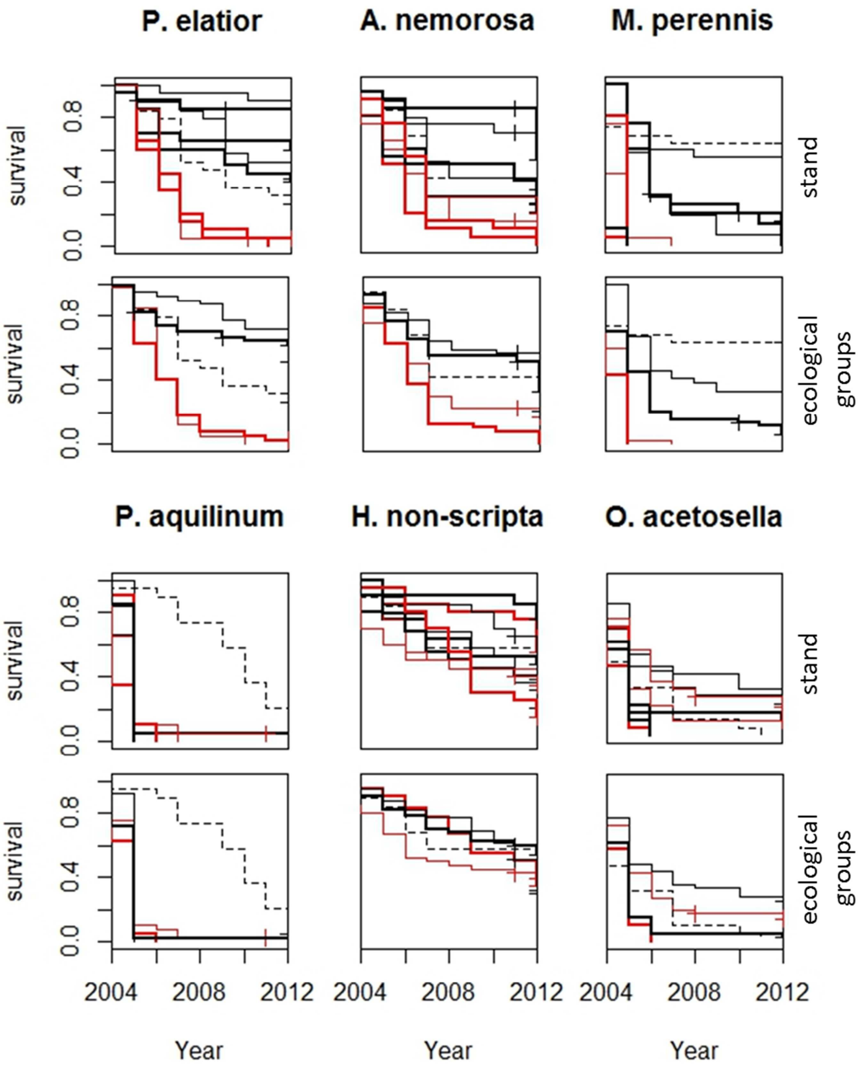


Figure 3.2 Survival of introduced ancient forest species in the different stands and ecological groups. Top row: acid intolerant and intermediate species, bottom row: acid tolerant species, left to right: increasing shade tolerance (Table 3.2). Black lines: moderately acidifying tree species, red lines: Acidifying tree species, thick lines: strongly shaded, thin lines: moderately shaded and dashed lines: clearcut.

The cover of *P. aquilinium* was only evaluated for the clearcut area because there were not enough data from the other stands. Cover of this species increased very rapidly on the clearcut area resulting in several square metres after 4-5 yr when competition with other competitors, mainly *U. dioica*, peaked. When the canopy closed again, the shade intolerant *P. aquilinium* declined and the total cover (Table 3.7) dropped below the initial level.

Table 3.6 Terms remaining in the model after model selection with p <0.01 and standard deviation of random intercept of GAMM models explaining the cover of introduced ancient forest species. Species sorted by acid tolerance and shade tolerance (Table 3.2)

Log(Cover)	N	∑df	Terms remained in the model	1|Stand s.d.
P. elatior	904	14.87	~ 1 + s(Year)*SHADE*ACIDITY + log(Plant weight)	0.45
A. nemorosa	532	7	~ 1 + Year*SHADE + ACIDITY	0.12
M. perennis[a]	370	8.39	~ 1 + s(Year)*SHADE	0.70
P. aquilinum[b]	94	3.87	~ 1 + s(Year)	
H. non-scripta	999	6	~ 1 + Year*SHADE	0.18
O. acetosella	301	8.85	~ 1 + s(Year)*SHADE	0.42

[a]: only moderately acidifying tree species included and [b]: only clearcut included

In half of the cases, the infrequently observed generative reproduction could not be modelled (Table 3.8). For the acid intolerant *P. elatior*, more grid points had flowering plants and seedlings in the moderately acidified stands than in the strongly acidified stands. Seedlings were sometimes found in large numbers in the moderately acidified stands, but recruitment was only successful in stands of *Fraxinus* and *Tilia*. Only five seedlings of *A. nemorosa*, were found, all in moderately acid stands. The intermediate acid tolerant *H. non-scripta* flowered regularly in stands of all kinds. However, seedlings of *H. non-scripta* were scarce and probably have not led to new adults. In other studies seeds germination of 5-20% is found (Baeten et al. 2009c; chapter 4 and 5). The difference with this study might be explained by the fact that this plant prefers cross-pollination (Corbet 1998). Corbet (1998) found that cross-pollinated flowers produce more and heavier seeds. The distance of 10m between the introduced plants in our study may have been too large to guarantee frequent cross-pollination. New adult plants are supposed to result from bulbs uprooted by molls, which was regularly observed. The shade tolerant *O. acetosella* flowered less in shaded stands. This species grew in a very patchy distribution and it was very difficult to assess the vegetative or generative origin of these new adults. Consequently, no results are presented for seedlings and seedling recruitment. In most cases new plants where found near the grid points but occasionally up to 5 m away from the nearest grid point. The acid intolerant *M. perennis* flowered and fruited frequently and independent of stand characteristics but no seedlings were found. Also other research has reported rare germination of this species (Mukerji 1936; Hutchings and Barkham 1976). It is unclear whether the two newly established individuals originated from seedlings or uprooted rhizomes. Sporulation of *P. aquilinium* was found only once on a plant with large leaves at the clearcut and new established plants were not found.

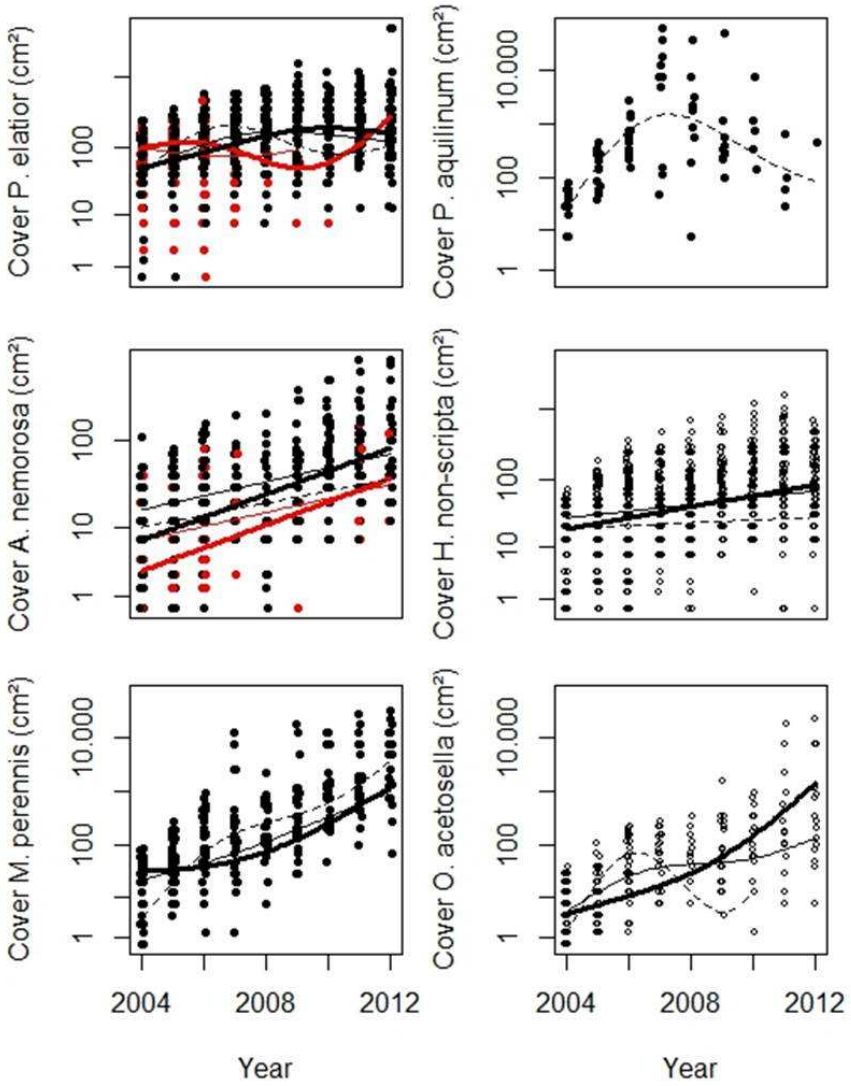


Figure 3.3 Plant cover (dots) and predictions for cover based on models in Table 3.5 (lines) for introduced ancient forest species with logarithmic scale on y-axis. Top row: acid intolerant and intermediate species, bottom row: acid tolerant species, left to right: increasing shade tolerance (Table 3.2). Black dots and lines: MOAC: moderately acidifying tree species, red dots and lines: ACID: Acidifying tree species, hollow circle and black lines: MOAC and ACID together, thick lines: strongly shaded, thin lines: moderately shaded and dashed lines: clearcut. For *Mercurialis perennis* only MOAC and for *Pteridium aquilinium* only clearcut is included.

Finally, for all species except *H. non-scripta* the total cover was higher in the moderately acid stands compared to the acid stands (Table 3.7). The two acid intolerant and one intermediate species increase in the moderately acid stands while the total cover decreased in nearly all acid stands. Total cover of *O. acetosella* increased strongly as its strong growth compensated for its high mortality but it also disappeared completely in three stands. In none of the stands *P. aquilinum* showed a net increase in total cover.

Table 3.7 Total cover within a stand of introduced ancient forest species 9 yr after introduction as a percentage of the initial total cover by ecological groups of stands (mean). Species sorted by acid tolerance and shade tolerance (Table 3.2)

N	ACID STSH (2)	ACID MOSH (2)	MOAC CLEAR (1)	MOAC STSH (3)	MOAC MOSH (2)
P. elatior	10%	0%	123%	472%	160%
A. nemorosa	0%	130%	632%	387%	158%
M. perennis	0%	0%	61 874%	469%	3353%
P. aquilinum	0%	0%	73%	3%	0%
O. acetosella	0%	4508%	0%	3916%	5848%

ACID: Acidifying tree species, MOAC: moderately acidifying tree species, MOSH: moderately shaded, STSH: strongly shaded, CLEAR: clearcut

In the models that explained the cover (Table 3.6), the flowering and seedlings (Table 3.8), the factor stand in the random intercept part only explained a small proportion of the deviance. Only in the model for the cover of *A. nemorosa* this factor included differences between stands within some of the a priori defined groups.

3.4 Discussion

This long term experiment revealed that tree species have a strong impact on the survival and population expansion of AFS in post-agricultural forest. This tree species effect can be explained as a combination of two main stress factors, i.e. the level of soil acidification and shade. When I included shade casting and soil acidification in the models, little remaining variability was explained by the tree species (cfr. Thomsen et al. 2005). Therefore, I assume that other tree species related factors were of minor importance for the success of the introduced AFS. While soil acidification was mainly important for survival, shade mainly affected growth. Interactions between soil acidification and shade casting were only rarely found. For both clonal and generative reproducing AFS, except *P. aquilinum*, suitable habitats for successful introduction or natural colonisation in post-agricultural forest could be created by selecting the appropriate tree species. Successful introduction of *P. aquilinium* probably requires a long period of high light availability that allows this species to generate a large network of rootstocks (den Ouden 2000). The tree species effects on AFS are discussed below specified for the following aspects: acidification, litter, shade and other tree species effects.

Table 3.8 Success rates in terms of flowering, producing seedlings and recruiting into new adults of introduced ancient forest species by ecological groups of stands, models (selection by p <0.01) and standard deviation of random intercept of GLMM models with n=198. Species sorted by acid tolerance and shade tolerance (Table 3.2)

	Terms remained in the model	1|Stand s.d.	ACID MOSH	ACID STSH	MOAC MOSH	MOAC STSH	MOAC CLEAR
Flowering							
P. elatior	~ 1 + ACIDITY	0.00	65%	55%	90%	85%	79%
A. nemorosa	/	/	3%	3%	26%	30%	0%
M. perennis	~ 1	0.69	3%	10%	21%	20%	16%
P. aquilinum	/	/	0%	0%	0%	0%	5%
H. non-scripta	~ 1	0.24	43%	70%	64%	67%	47%
O. acetosella	~ 1 + SHADE	0.00	18%	5%	28%	5%	21%
Seedling							
P. elatior	~ 1 + ACIDITY	0.93	10%	5%	79%	52%	21%
A. nemorosa	/	/	0%	0%	5%	5%	0%
M. perennis	/	/	0%	0%	3%	0%	5%
H. non-scripta	/	/	3%	10%	10%	12%	0%
Seedling recruitment							
P. elatior	/	/	0%	0%	13%	28%	16%
H. non-scripta	/	/	3%	8%	5%	3%	0%

/: models did not lead to convergence

Tree species that caused soil acidification below pH-H_2O 4.2 negatively affected the survival, vegetative and generative expansion of AFS listed as acid intolerant according to indicator values. By contrast, AFS listed as acid tolerant species seemed not to benefit by soil acidification. For example, Mg deficiency disease (yellowing between leaf veins) was found on *A. nemorosa* and *P. elatior* during the first years in the acid and shaded stands of *Fagus* and *Quercus*. This finding could indicate that soil acidification is explanatory for the failure of these species in strongly acidified stands. Tree species affect the availability of Calcium (Ca) and Mg, rather than the total soil concentration (Finzi et al. 1998). Increasing pH-H_2O with or without adding Ca and Mg had similar effects on the germination of AFS, which clarified that bio-availability instead of soil concentration was limiting (Falkengren-Grerup and Tyler 1993). Antagonism between Mg and increased availability of Al may explain the failure of plant species under acidifying tree species (Weber-Blaschke et al. 2002). A soil pH-H_2O value of 4.2 seems to be a threshold for acid intolerant plants (cf. Brunet and Neymark 1992; Falkengren-Grerup and Tyler 1993). I conclude that soil acidification is only tolerated by a limited number of acid tolerant AFS while moderately high pH-H_2O levels are suitable for both acid tolerant and intolerant species.

Besides chemical effects of soil acidification also mechanical effects of litter accumulation probably are a factor in the trees species effect. Since litter accumulation is correlated with soil acidification, it is not possible to discriminate between these two factors. However, in litter removal experiments forest grasses show reduced biomass production when litter depth increases while typical forest perennials, like *H. non-scripta*, *A. nemorosa* and *L. galeobdolon* are less sensitive (Sydes and Grime 1981a). As a consequence, the competitive relation between both groups is affected (Sydes and Grime 1981b). Staaf (1992) even found negative effects of litter removal on adult introduced AFS. In contrast, litter removal has beneficial effects for seedling recruitment for most forest plants (Eriksson and Ehrlén 1992; Eriksson 1995). None of the species investigated by Eriksson (1995) was favoured by litter, *A. nemorosa* was one of the few species capable to germinate in litter. I also noted that not only the amount of litter, but also the tree species-specific leaf size was important. For example, *H. non-scripta* was often found entangled within the large leaves of *Q. rubra*, which could explain the relative low survival rate in this stand as compared to the *F. sylvatica* stand with similar soil and light conditions. Leaf litter thus might have both beneficial and negative effects: litter could hamper the initial germination and recruitment of AFS while in later stages a litter layer could provide a competitive advantage to some AFS, as long as they can cope with the associated acidity.

The third important factor explaining tree species effects is the specific level of shade casting ability. Moderately shaded stands had higher survival rates for both shade tolerant and intolerant species. This can be explained by the fact that both shade tolerant and intolerant species need sufficient light for their initial establishment. Further, shade casting is negatively correlated with herb layer cover which might be a better explanation for the sensitivity of some species. For example, the shade tolerant *O. acetosella* requires moist soils and high relative humidity (Packham and Willis 1976; Packham 1978). Due to the low vegetation cover in strongly shaded stands, the small and fragile transplants of this species were more exposed to dry and windy weather conditions than in stands with intermediate light levels, where sheltering vegetation was present. The same may hold for the shade tolerant *M. perennis*. In contrast to the survival, the growth of the introduced AFS was in general better in deeply shaded stands. This could be explained by the spring phenology of most of the species, completing most of the life-cycle before the leaves of trees emerge, whereas light-demanding competitive herbs emerge later and are reduced by a high canopy cover. As a result shade-tolerant forest plants benefit from the absence of root and light competition by light-demanding competitors and consequently grow better in stands with low light levels (cf. De Keersmaeker et al. 2004).

Baeten et al. (2009b) found a declining population size of introduced *P. elatior* in post-agricultural Poplar stands explained by a lower seedling recruitment and lower adult longevity in the post-agricultural forest compared to the ancient forest. In our study the longevity is very similar in our moderately shaded and moderately acid *Fraxinus* stands, but recruitment compensated the losses in both stands. Probably, our *Fraxinus* stands generated just enough shade to avoid a dominance of competitors like *U. dioica* while this was not the case in the study of Baeten et al. (2009b). As a result, survival of *P. elatior* increased with age in our study (cf. Ehrlén and Eriksson 2000) while it decreased in the study of Baeten et al. (2009b). This again illustrates that light availability could be highly important to control the competitors with elevation of bio-P.

On the clearcut, species showed both higher and lower survival, mainly depending on their competitive nature rather than their shade tolerance. More competitive forest species, like *P. aquilinium* and *M. perennis* performed better on the clearcut while the two hemicryptophytes which depend on seedling recruitment for expansion, *P. elatior* and *O. acetosella* did worse than in other moderately acid stands. Verheyen and Hermy (2004) concluded that hemicryptophytes need gaps in the vegetation for seedling establishment since they are summer-green in contrast to many spring ephemeral geophytes that avoid competition. From our study, I can conclude that open gaps can only support establishment of seed depended hemicryptophytes when vegetative spreading competitors, like *U. dioica*, cannot establish, for example when gaps close quick enough to prevent competitors of becoming dominant. Furthermore, AFS have a higher turnover on P elevated sites making generative species even more dependent on seedling recruitment. Also De Keersmaeker et al. (2011b) demonstrated that clear cutting a Poplar stand facilitated the establishment of new forest plant species, which was explained by the temporal increased light levels and local litter removal. This effect was most successful on locations where the shrub layer resprouted vigorously. Similar to our study, also De Keersmaeker et al. (2011b) found that the newly establishing forest species had a more competitive nature (e.g. ferns and grasses). I assume that temporary gaps on soil with high P availability will promote species with a more competitive strategy. Therefore, gaps need to be smaller and close quicker on P enriched sites to be suitable for the recruitment of AFS. The high recruitment of *P. elatior* seedlings in the shaded stands demonstrates that at least some AFS can also recruit in strongly shaded stands. It is, however, unclear how many other forest species can successfully germinate in the spring before leafing out of the canopy.

The increased soil N concentration in the *Alnus* stand is explained by a symbiosis of this tree with N-fixating bacteria, while the low N concentration in the *Robinia* stand might indicates low or absent N-fixating in this particular stand. I could not detect any strong differences concerning the introduced plants between the *Alnus* stand with high soil N concentration and the other stands and, therefore, assume that the vegetation was not N limited in any stand. This is, furthermore, supported by the fact that N deposition in the study area is still above thresholds of species-rich forest habitats (Overloop et al. 2011). The availability of P depends on the soil pH-H_2O (Stevenson and Cole 1999) and consequently, is related to the tree species. However, the difference in bio-P concentration between the stands shows no link with the soil pH-H_2O. Furthermore, volume or basal area as a proxy for P fixation in wood (De Schrijver et al. 2012b) does not explain the bio-P concentrations in our experiment. Furthermore, I see no effect on the success rates of the introduced plants by the differences in bio-P, again likely explained by the fact that no stands were P limited as the P levels in Mortagne forest are two to seven times higher than in ancient forest (De Keersmaeker et al. 2004; Baeten et al. 2010).

From this study, I can conclude that tree species induced stress factors can be an essential element in the success of forest restoration on eutrophicated farmland, both for natural and assisted colonisation. However, the type and level of stress depends on the species targeted and specific life stage of the species (cf. Drayton and Primack 2012). More precisely, the initial establishment phase and the subsequent colonisation are influenced differentially (cf. Staaf 1992; Gustafsson and Ehrlén 2003; but see Verheyen and Hermy 2004). Specific management interventions could, furthermore, optimize the light conditions for each stage in the plant colonisation process.

The choice of the most appropriate tree species depends on the species that can potentially colonise the site considered for restoration. Since soil pH-H_2O levels above the critical value for Al-toxicity (pH-H_2O 4.2) support both acid tolerant and intolerant species and as most of the AFS are acid intolerant (Hermy et al. 1999), maintaining a high pH-H_2O level, by selecting an appropriate tree species is advised.

Lower levels of stress by a more open canopy and litter removal (for example through thinning or coppicing) might be beneficial as site preparation in the initial face of an introduction or natural recruitment (cf. Menges 2008) while stands are kept strongly shaded and undisturbed afterwards to suppress competitors and expand the populations of the AFS (De Keersmaeker et al. 2004). Finally, cyclic alternations by for example coppicing could generate frequent temporary gaps and intermediate strongly shaded sites which could optimise AFS colonisation further.

4

Tree species effects on the recruitment of forest herbs: a pot experiment

After: Thomaes, A., De Keersmaeker, L., De Schrijver, A., Vandekerkhove, K., Verschelde, P. & Verheyen, K. 2011. Can tree species choice influence recruitment of ancient forest species in post-agricultural forest? Plant ecology, 212: 573-584

Abstract

Germination and establishment of AFS are bottlenecks in forest habitat restoration. I hypothesized that tree species can influence these processes on acidification sensitive soils through their effects on the soil. Seeds of seven AFS were sown in soil collected in a post-agricultural forest under four different tree species, notably *A. pseudoplatanus*, *A. glutinosa*, *F. sylvatica* and *P.* x *canadensis*. Germination of four species was affected by tree species, while only *H. non-scripta* was indifferent and germination of *A. nemorosa* and *P. quadrifolia* was unsuccessful. The acid intolerant *P. elatior* only germinated in the soil of *Populus* but also the acid tolerant *L. periclymenum* and *S. holostea* performed best in the soil of *Populus* and worst in the soil of *Alnus*. *L. galeobdolon* preferred the soil of *A. pseudoplatanus*. Germination of competitive seed bank species was much more successful in the soil of *Populus* than in soil of other tree species. From our results I deduce that tree species selection can affect germination of AFS. Forest managers should be aware of the effects of tree species on the colonisation chances of AFS and favour trees with good decomposable litter especially on acidification sensitive soils.

4.1 Introduction

Germination and seedling survival of ancient forest plants plays an important role in the colonisation of post-agricultural forests. Colonisation of young forest adjacent to ancient forest depend both on vegetative and generative reproduction (Brunet et al. 2000; Singleton et al. 2001; Honnay et al. 2002b). However, germination and seedling survival of AFS has been found to be a bottleneck for colonisation (Ehrlén and Eriksson 2000; Verheyen and Hermy 2004). Colonisation of isolated post-agricultural forests happens rarely, but completely relies on diaspores (Dzwonko and Loster 1988).

In chapter 2 and 3, the general patterns of tree species effects on AFS colonisation was studies, respectively in a chronosequence and common garden with different species. Here, I focused on the tree species effect through soil on germination and recruitment. I performed a pot experiment to study whether different tree species can influence the recruitment success of AFS in post-agricultural forests. The tree species selected for our study were known to have a differential effect on soil characteristics. Likewise, I selected AFS with different ecological needs. This study was performed with slightly acid to neutral Luvisols since these are: (1) sensitive to acidification and tree species have a profound role in the development of these soils (van Oijen et al. 2005), (2) ancient forests on these soils are the most threatened (Foster 1992; Kirby and Watkins 1998; De Keersmaeker et al. 2004) and (3) most AFS occur on these soils (Hermy et al. 1999). I assume that tree species work as ecosystem engineers (Jones et al. 1997) influencing the potential for AFS colonisation in post-agricultural forest and hypothesize that germination and seedling survival of AFS in soil collected under these different tree species will be different.

4.2 Material & Methods

4.2.1 Experimental set-up

The pot experiment was performed with soil from Mortagne forest (see chapter 1). Based on the results of Dossche (1998), see Figure 1.3, four stands encompassing the range of soil characteristics were selected: *A. pseudoplatanus, A. glutinosa, F. sylvatica* and *P.* x *canadensis* (Acer8, Alnus5, Fagus6, Populus8, Table 1.1). Mineral topsoil (0-10 cm) was collected at two central locations in each of the four selected stands in September 1999 (i.e. 27 year after afforestation). The soil was mixed, sieved to remove roots, branches and leaves and distributed over plastic flowerpots. Each pot measured 12 cm in diameter and contained approximately two kg of fresh soil.

Seven AFS were selected from Table 3.3: *A. nemorosa*, *L. galeobdolon*, *L. periclymenum*, *P. quadrifolia*, *P. elatior*, *H. non-scripta* and *S. holostea* (Table 4.1). Only the last species was present in some parts of Mortagne forest but absent in the plots where soil was collected. The selected AFS represent a wide range of tolerance towards soil acidity: *L. galeobdolon*, *P. quadrifolia* and *P. elatior* are found on more or less neutral soils as they are acid intolerant. *H. non-scripta* and *S. holostea* are indicators of moderately acid soils and are intermediate tolerant. *A. nemorosa* is also intermediate tolerant or indifferent towards soil acidity according to Ellenberg et al. (1992). *L. periclymenum* is strongly acid tolerant. Propagules were collected in an ancient forest, the Aelmoeseneie forest (50.9° N, 3.88° E, Belgium), except seeds of *H. non-scripta* which were collected in another ancient forest (Raspaille forest, 50.77° N, 3.93° E, Belgium). Propagules were collected when they were fully ripened during spring and summer of 1999 and stored dry, indoor and in darkness during the rest of the summer.

Table 4.1 Seeded species with number and mean mass of the introduced propagules compared with mean propagule mass in the LEDA traitbase (Kleyer et al. 2008). AFS are ordered by increasing propagule weight according to the LEDA traitbase.

Species	Number of propagules /pot	Mean weight /propagule (mg) Introduced propagule	LEDA
P. elatior	80	0.7	0.3
A. nemorosa	10	1.8	2.1
L. galeobdolon	10	2.7	2.4
S. holostea	10	2.9	2.6
P. quadrifolia	100	0.8	4.5[a]
H. non-scripta	10	4.1	5.5
L. periclymenum	20	6.6	5.6

[a]: Based on Jacquemyn et al. (2008) and median value in the LEDA traitbase.

A pot experiment was preferred as many factors that are not related to tree species effects can be excluded, resulting in higher germination. To have a general idea of the in situ success of seeds, a small seed experiment was also performed in situ. In each of the four stands, seeds were sown in one representative plot. The litter layer was removed and seeds (double amount of the flowerpots) of each species were sown in subplots of 10x10 cm. Afterwards the litter layer was replaced.

The experiment included 256 flowerpots: eight replicates for each combination of tree species (4, referred to as TS) and AFS (7) or control (to study the seed bank). propagule mass for each pot was measured before sowing in September 1999. The propagule mass and the number of propagules sown of each AFS are listed in Table 4.1. For species with lower propagule mass, higher numbers of propagules were sown as lower germination is expected for species with low propagule mass (cf. Baskin and Baskin 2001). Pots were placed directly outside in a mixed stand of *Q. robur* and *F. sylvatica* in the Aelmoeseneie forest to condition under natural temperature, shade and humidity. Pots were placed on a wooden frame to prevent interaction with soil ground water and were protected against birds and rodents using a net. The soils in the pots remained moist throughout the research and hence, did not need to be watered additionally. The position of the pots was regularly changed randomly to avoid differences in light or water. Seedlings germinating from the seed bank were removed to eliminate competition, except from the control pots.

The numbers of seedlings were counted six times: 8 November, 3 December 1999, 11 February, 29 February, 18 March and 17 May 2000. Seedlings of *L. galeobdolon* (since it only started to germinate in May 2000) were checked once more on 12 July 2000 and *A. nemorosa* and *P. quadrifolia* (which failed to germinate) on 12 July 2000 and 8 April 2001. The number of leaves of each seedling was recorded to distinguish between new and surviving seedlings since the previous observation.

4.2.2 Soil analyses

Soils were analysed both before and after the pot experiment, but since results were comparable (except P, see discussion), I only present the data of soil collected afterwards. After the experiment, I selected eight flowerpots from each TS randomly for soil analysis. Samples were analysed for pH-KCl (determined from a solution of 10 g soil and 25 ml 1 N KCl using a glass calomel electrode, Van Ranst et al. 1999). The total amount of N was determined with a modified Kjeldahl method, using Se as a catalyst (Van Ranst et al. 1999). Furthermore, 5 g dry soil was extracted in 100 ml ammonium lactate solution (9.01 ml lactic acid (88%) + 18.75 ml acetic acid (99%) + 7.75 ml NH_4-acetate diluted to 1 l) according to a modified method of Egnèr et al. (1960) and afterwards analysed on (1) Potassium (K), Ca and Mg using Atomic Absorption Spectrometry (Varian, SpectrAA-220) and (2) P by means of a spectrophotometer (Varian, Cary 50) according to the colorimetric method of Scheel (1936) with molybdenum vanadate as colour reagent.

4.2.3 Data analyses

Three variables were derived from the experiment: (1) number of seedlings (N_{seedl}), which is the total number of emerged seedlings accumulated over all observations; (2) Germination (GERM), which is N_{seedl} divided by the number of introduced seeds and (3) recruitment or survival (SURV), which is the number of seedlings present at the last observation divided by N_{seedl}.

By using the S+ routine, logistic regressions were created with the interaction between TS and AFS as variable, for AFS (GERM and SURV, excluding *P. quadrifolia*, see results) and TS for seed bank (N_{seedl} and SURV). For N_{seedl} of seed bank Poisson regression was used instead of logistic regression. Differences between tree TS were analysed by comparing 95% confidential intervals of the predicted values corrected for multiple testing with Bonferroni correction.

Differences between TS for the period at which the first seedlings and the highest number of seedlings were observed were tested using Kaplan-Meier survival analyses entering the periods numbered from 1 to 8. In case an overall significant difference was found, four additional tests were performed, comparing one TS with the three other TS together using Bonferroni correction for the p-value.

Including seed mass as a co-variable in the logistic regressions and Kaplan-Meier analyses did not resulted into a significantly improved model. By consequence, the variable was not included for the statistical analyses presented here. Two-way ANOVA test was applied to the soil characteristics. All statistics were conducted with S-Plus 6.2 Professional.

4.3 Results

All soil chemistry variables were significantly different between the four TS (Table 4.2). *Populus* had the highest pH-KCl and soil base saturation (Ca+Mg+K), followed by *Fagus*, *Acer* and *Alnus*. As expected, the soil of the N-fixing *Alnus* had a higher N content. Bio-P was significantly higher in the soil of *Acer*.

None of the AFS emerged from the seed bank in the control pots or in pots where other species were sown. This indicates the absence of a viable seed bank of the AFS which would influence the results of the experiment.

Table 4.2 Results of ANOVA on the chemical characteristics of flowerpot soils (mean±s.e.), compared between tree species. Equal letters in superscript indicate values which are not significantly different between tree species.

	$F_{3,28}$	p	*Alnus*	*Acer*	*Fagus*	*Populus*
pH-KCl	143.7	***	3.2 ± 0.01^{a}	$3.3 \pm 0.02^{a,b}$	3.4 ± 0.01^{b}	4.3 ± 0.08^{c}
Ca ($mg.kg^{-1}$)	300.0	***	377 ± 24^{a}	431 ± 10^{a}	442 ± 25^{a}	$1\,460 \pm 58^{b}$
Mg ($mg.kg^{-1}$)	395.9	***	49 ± 2^{a}	50 ± 1^{a}	53 ± 1^{a}	105 ± 2^{b}
K ($mg.kg^{-1}$)	87.4	***	119 ± 1^{a}	170 ± 3^{b}	154 ± 9^{b}	231 ± 3^{c}
Kj-N ($mg.kg^{-1}$)	25.2	***	3823 ± 231^{c}	3023 ± 130^{b}	2312 ± 121^{a}	2253 ± 31^{a}
Bio-P ($mg.kg^{-1}$)	31.3	***	44 ± 3^{a}	78 ± 2^{b}	35 ± 6^{a}	35 ± 1^{a}

Kj-N: Kjeldahl nitrogen; Bio-P: Bio-available P and ***: $p < 0.001$

Emergence of *P. quadrifolia* failed and GERM of *A. nemorosa* amounted only in the second year to 4% (Table 4.3). Therefore, a TS effect on *P. quadrifolia* could not be tested and for *A. nemorosa* should be carefully evaluated.

The regressions for GERM and NSeedl explained more than 90% of data deviance, while regressions for SURV covered 64 and 72% for AFS and seed bank respectively (Table 4.4). GERM of four species (*L. galeobdolon, L. periclymenum*, *P. elatior* and *S. holostea*) was TS dependent while only GERM of *H. non-scripta* was TS independent (Table 4.3). Germination of *L. periclymenum* and *S. holostea* was significantly higher in the soil of *Populus* (Table 4.3). *P. elatior* only germinated in the soil of *Populus*, since there is no variance for the other TS significance cannot be proven in this set up. Germination in the soil of *Alnus* was lower for *L. galeobdolon* and *L. periclymenum*. *L. galeobdolon* is the only AFS for which GERM was high in the soil sampled under *Acer*. There was a lower SURV of *L. periclymenum* and *S. holostea* (for this last species not significant) in soils of *Alnus* and *Populus* compared with soils of the two other TS (Table 4.3).

In the in situ seed sowing experiment, no seedlings were found in the stand of *Acer* and *Alnus*. The plot in the *Populus* stand was destroyed by a mountain bike trail. In the *Fagus* stand one seedling was found of *H. non-scripta* (5% recruitment), one of *S. holostea* (5%) and seven of *L. periclymenum* (18%).

A relation between TS and period of emergence was found for three AFS (Table 4.5). Seedlings of *P. elatior* only emerged in the soil of *Populus* (Figure 4.1). The highest number of seedlings of *L. periclymenum* peaked later in time in soil of *Alnus* and *H. non-scripta* germinated later in soil of *Populus* (both nearly significant).

Table 4.3 Mean value of germination (GERM) and survival (SURV) compared between tree species. Equal letters in superscript indicate values which are not significantly different between tree species based on logistic and Poisson regression (Table 4.4). Tree species are ordered by increasing topsoil pH-KCl and AFS by decreasing acid tolerance (Table 3.3).

Mean±s.e. (n)	*Alnus*		*Acer*		*Fagus*		*Populus*	
L. periclymenum GERM	0.43 ± 0.06^{a}	(8)	$0.58\pm0.08^{a,b}$	(8)	$0.63\pm0.04^{a,b}$	(8)	0.66 ± 0.05^{b}	(8)
SURV	0.51 ± 0.08^{a}	(8)	0.83 ± 0.07^{b}	(8)	0.90 ± 0.04^{b}	(8)	0.57 ± 0.06^{a}	(8)
H. non-scripta GERM	0.14 ± 0.04^{a}	(8)	0.06 ± 0.02^{a}	(8)	0.14 ± 0.03^{a}	(8)	0.10 ± 0.06^{a}	(8)
SURV	0.71 ± 0.15^{a}	(7)	1.00 ± 0.00^{a}	(5)	0.81 ± 0.13^{a}	(8)	1.00 ± 0.00^{a}	(4)
A. nemorosa GERM	$0.\pm0.00^{a}$	(8)	0.06 ± 0.02^{a}	(8)	0.05 ± 0.02^{a}	(8)	0.05 ± 0.02^{a}	(8)
SURV	-	(0)	0.80 ± 0.20^{a}	(5)	1.00 ± 0.0^{a}	(4)	1.00 ± 0.00^{a}	(4)
S. holostea GERM	0.24 ± 0.04^{a}	(8)	0.26 ± 0.05^{a}	(8)	0.18 ± 0.05^{a}	(8)	0.56 ± 0.11^{b}	(8)
SURV	0.55 ± 0.14^{a}	(8)	0.68 ± 0.16^{a}	(8)	0.68 ± 0.18^{a}	(7)	0.27 ± 0.09^{a}	(8)
L. galeobdolon GERM	0.04 ± 0.02^{a}	(8)	0.30 ± 0.07^{b}	(8)	$0.11\pm0.04^{a,b}$	(8)	0.05 ± 0.03^{a}	(8)
SURV	1.00 ± 0.00^{a}	(3)	0.94 ± 0.06^{a}	(7)	0.83 ± 0.17^{a}	(6)	0 ± 0.00^{a}	(3)
P. quadrifolia GERM	0±0.00	(8)	0±0.00	(8)	0±0.00	(8)	0±0.00	(8)
SURV	-	(0)	-	(0)	-	(0)	-	(0)
P. elatior GERM	0 ± 0.00^{a}	(8)	0 ± 0.00^{a}	(8)	0 ± 0.00^{a}	(8)	0.76 ± 0.05^{a}	(8)
SURV	-	(0)	-	(0)	-	(0)	0.86±0.07	(8)
Seed bank N_{Seedl}	1.8 ± 0.5^{a}	(8)	0.5 ± 0.2^{a}	(8)	0.8 ± 0.3^{a}	(8)	21.1 ± 3.3^{b}	(8)
SURV	0.48 ± 0.19^{a}	(7)	0.25 ± 0.25^{a}	(4)	0.60 ± 0.24^{a}	(5)	0.96 ± 0.04^{a}	(8)

-: missing values due to absent germination

Table 4.4 Results of the logistic regression of germination (GERM) and survival (SURV) and Poisson regression for NSeedl of seed bank.

Model	df	% deviance	F	p
AFS (excluding *P. quadrifolia*)				
GERM ~ TS x AFS	24,168	92.5	99.25	***
SURV ~ TS x AFS	20,107	63.6	9.73	***
Seed bank				
NSeedl ~ TS	4,28	93.4	112.74	***
SURV ~ TS	4,20	72.3	11.95	***

***: $p < 0.001$

The seedlings that germinated from the seed bank were mainly *Betula* spp., *Poaceae*, *Juncaceae*, *Lamiaceae* and *Asteraceae* such as *Cirsium* spp. and *Taraxacum* spp.. Germination of the seed bank was also TS dependent, with higher N_{Seedl} (Table 4.4) and earlier emerging seedlings (Table 4.5, Figure 4.1) in the soil from *Populus*.

4.4 Discussion

Our results show a clear TS effect on germination and seedling survival from AFS in post-agricultural forests. This effect was present despite the fact that only the topsoil was used thus excluding a potential effect through the light regime or a physical effect of the litter layer (Barbier et al. 2008). Germination and survival of four of the tested AFS appears to be tree species dependent. Germination of most of the species followed the soil pH-KCl gradient created by the different tree species.

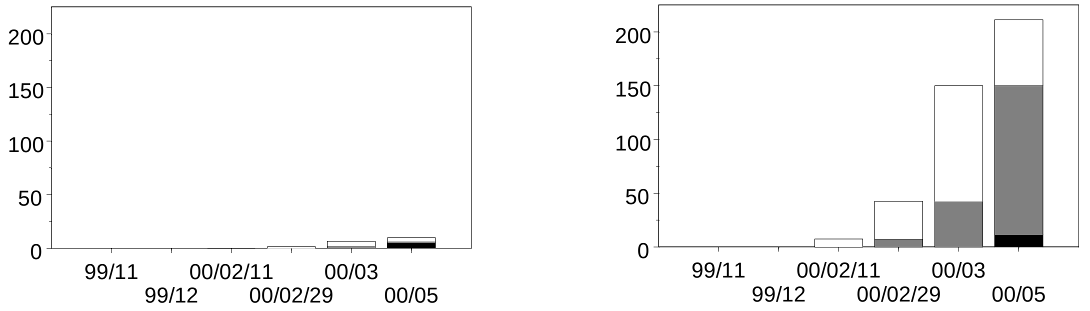
Table 4.5: Results of the Kaplan-Meier survival analyses for the period with first seedlings (tF) and the highest number (tH) of seedlings. Underlined results are significantly different from the other tree species grouped together. Tree species are ordered by increasing topsoil pH-KCl and AFS by decreasing acid tolerance (Table 3.3).

		Likelihood ratio test (df= 3)	P	Mean period±s.e. (n) ^{a}			
				Alnus	*Acer*	*Fagus*	*Populus*
L. periclymenum	tF	0.00	1.00	3.0±0.0 (8)	3.0±0.0 (8)	3.0±0.0 (8)	3.0±0.0 (8)
	tH	6.84	0.08	3.1±0.1 (8)	3.5±0.3 (8)	4.0±0.3 (8)	4.0±0 (8)
H. non-scripta	tF	7.32	0.06	4.6±0.4 (7)	5.0±0.5 (5)	4.8±0.5 (8)	5.9±0.1 (4)
	tH	6.68	0.08	5.1±0.3 (7)	5.0±0.5 (5)	5.4±0.4 (8)	6.0±0.0 (4)
A. nemorosa	tF	10.3	0.02*	8.0±0.0 (0)	7.8±0.2 (5)	8.0±0.0 (4)	8.0±0.0 (4)
	tH	10.3	0.02*	8.0±0.0 (0)	7.8±0.2 (5)	8.0±0.0 (4)	8.0±0.0 (4)
S. holostea	tF	4.65	0.20	2.0±0.5 (8)	2.5±0.6 (8)	3.3±0.7 (7)	2.0±0.3 (8)
	tH	6.06	0.11	3.1±0.4 (8)	4.1±0.4 (8)	4.1±0.5 (7)	3.8±0.2 (8)
L. galeobdolon	tF	6.13	0.11	6.6±0.2 (3)	6.1±0.1 (7)	6.3±0.2 (6)	6.6±0.2 (3)
	tH	5.42	0.14	6.6±0.2 (3)	6.3±0.2 (7)	6.4±0.2 (6)	6.6±0.2 (3)
P. elatior	tF	32.3	0.00***	6.0±0.0 (0)	6.0±0.0 (0)	6.0±0.0 (0)	4.0±0.0 (8)
	tH	32.3	0.00***	6.0±0.0 (0)	6.0±0.0 (0)	6.0±0.0 (0)	5.4±0.2 (8)
Seed bank	tF	25.7	0.00***	5.0±0.3 (7)	5.6±0.2 (4)	5.6±0.2 (4)	3.5±0.2 (8)
	tH	5.07	0.17	5.3±0.2 (7)	5.6±0.2 (4)	5.6±0.2 (4)	5.8±0.2 (8)

^{a}: period 1= 8 Nov.; 2= 3 Dec.1999; 3= 11 Feb.; 4= 29 Feb.; 5= 18 March; 6= 17 May; 7= 12 July2000 and 8= 8 April 2001. *: $p < 0.05$ and ***: $p < 0.001$.

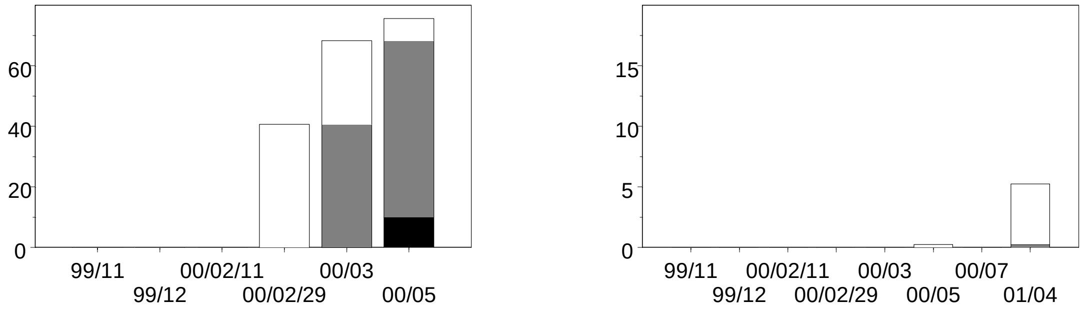
a: Number of seedlings from the seed bank (all except *Populus*)

b: Number of seedlings from the seed bank (only *Populus*)



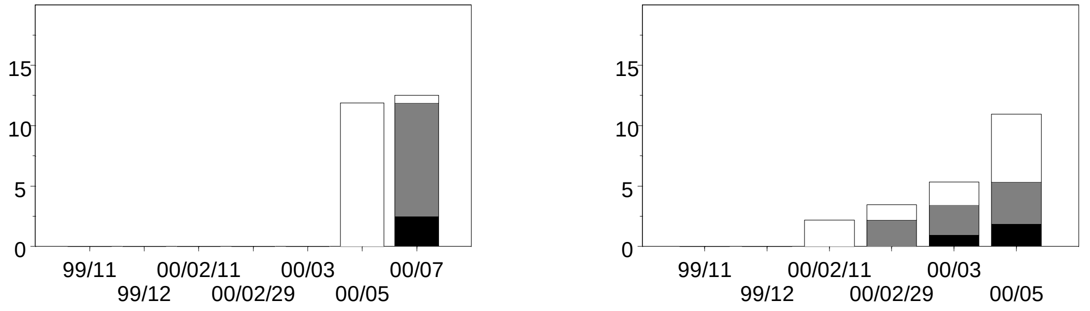
c: Seedlings of *Primula elatior* (%) (only *Populus*)

d: Seedlings of *Anemone nemorosa* (%) (all except *Alnus*)



e: Seedlings of *Lamium galeobdolon* (%) (all tree species)

f: Seedlings of *Hyacinthoides non-scripta* (%) (all tree species)



g: Seedlings of *Stellaria holostea* (%) (all tree species)

h: Seedlings of *Lonicera periclymenum* (%) (all tree species)

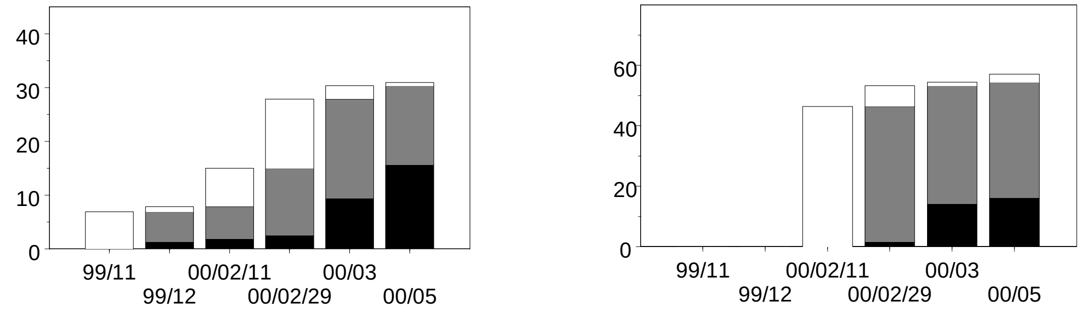


Figure 4.1 Barplots of the seedling/seed ratio (%, c-h) and exact numbers of seedlings for the seed bank (a-b). White bars: newly emerged; grey: surviving and black bars: died seedlings since previous observation. Different scales are used on Y bars and *A. nemorosa* and *L. galeobdolon* have a different time line. Separate plots for certain tree species are based on significant differences by Kaplan-Meier survival analyses (Table 4.5).

A. nemorosa showed low germination and *P. quadrifolia* failed to germinate. Germination of *A. nemorosa* in other studies varied from 0 up to 91% (Grime et al. 1981; Shirreffs 1985; Falkengren-Grerup and Tyler 1993; Verheyen and Hermy 2004; De Frenne et al. 2010). Our low result is probably explained by the dry storage of the propagules (Shirreffs 1985; Baskin and Baskin 2001). Seed masses of *P. quadrifolia* in this study were five times lower than data from LEDA traitbase and Jacquemyn et al. (2008) (Table 4.1) indicating that our seeds might have been unfertilized. Ideally a tetrazolium test had been performed to verify the viability. It is at least remarkable that both species are mainly clonally spreading species which are less dependent on fertile seeds.

It is unclear which soil factors explain our tree species-understory relation since potentially many factors can explain this relation. Because only four tree species are tested, I cannot relate the results to the individual soil variables since these strongly cluster to the individual tree species. From the soil variables only the pH-KCl gradient follows the same pattern as the germination of most of the AFS. Therefore, the tree species effect through soil seems to be a pH related effect. Soil pH is found to be an important factor in other studies to explain the tree species-understory relation (Barbier et al. 2008). Differences in the understory under different tree species were mainly explained by differing soil pH values (Augusto et al. 2003; Thomsen et al. 2005). More acidophilous understory species are found under tree species which support a more acid soil and vice versa (Aubert et al. 2004). Furthermore, sowing experiments with 13 AFS (Falkengren-Grerup and Tyler 1993) and 4 rare forest grasses (Brunet and Neymark 1992) revealed a positive reaction for all species to an increased pH-H20 by means of a carbonate treatment. Acid tolerant species, *Deschampsia flexuosa* (L.) Trin. and *L. pilosa*, showed only a small positive response to the pH-H2O gradient from 3.3 to 5.2, while acid sensitive species, *Bromus ramosus* Huds. subsp. *benekenii* (Lange) Schinz et Thell., *B. ramosus* subsp. *ramosus*, *C lutetiana*, *Festuca altissima* All., *Galium odoratum* (L.) Scop., *Hordelymus europaeus* (L.) Harz, *L. galeobdolon* and *Sanicula europaea* L. failed to germinate below a certain pH-H2O threshold (between 3.9 to 5.0 depending on the species). In our research the acid sensitive species *P. elatior* germinated only in the soil of *Populus* with a pH-KCl of 4.3. Brunet and Neymark (1992) and Falkengren-Grerup and Tyler (1993) used both $SrCO_3$ and $CaCO_3$+$MgCO_3$ to illustrate that the effect is induced by the pH-$H_2$0 rather than the addition of Mg and Ca.

From the species that have their optimum in acidic forest types (Table 4.1) two, *L. periclymenum* and *S. holostea*, germinated better in soils with a higher pH-KCl. The third acid tolerant species, *H. non-scripta*, has reacted indifferently to tree species and related soil acidity. This probably means that the fundamental niche differs from the realised for the first two species (Hutchinson 1957). This seems logic since our results show higher germination of competitors in the soils with a higher pH-KCl as well. This underlines the acid tolerant nature rather than acidophilous nature of these plant species. In conclusion for the germination both acid tolerant as acid sensitive species are not disfavouring tree species with a good decomposable litter. This is in agreement with the experiments of Falkengren-Grerup and Tyler (1993) and Brunet and Neymark (1992) where none of the species, including several acid tolerant species, have been disfavoured by a high pH-H_20.

It has been hypothesized that high P levels negatively affect later plant development stages of *P. elatior* (Baeten et al. 2009b). We, however, did not find evidence that high P limited or hampered germination when competition was excluded but in our study all P levels were relatively high. In contrast, germination of *L. galeobdolon* was even highest on the *Acer* soils containing the highest levels of bio-P. This result is in accordance with the study of Packham (1983) who found a higher occurrence of *L. galeobdolon* on P-enriched sites. Furthermore, Falkengren-Grerup et al. (1994) found an enhanced growth of *G. odoratum, L. galeobdolon* and *S. holostea* with increasing P-availability in a fertilisation experiment. High bio-P levels are probably more important in their effect on competitors and the altered relationship between these and AFS (De Keersmaeker et al. 2004). I have not found a plausible explanation for the high bio-P levels in the soil of *Acer*, there is no literature confirming high bio-P levels for *Acer* and there is also no historical difference of this stand which can explain the high bio-P level. Therefore, I assume it is due to a local artefact. In a previous study at the same site (Dossche 1998) bio-P levels in the soil of *Acer* were comparable to other tree species.

Tree species differ not only in soil development but also in thickness of litter and in light conditions affecting the understory (Barbier et al. 2008) which was not studied in this pot experiment. The in situ experiment revealed that at least three out of seven species can still recruit in plots with thick litter layers and dark overstory. However high seed rains might be needed to successfully recruit and colonise such a plot. *Quercus* spp. and *F. sylvatica* may be rather equal in litter and soil development but differ in light conditions, while *A. glutinosa* may create equally acid soils but its litter is better degradable from the previous two species (Dossche 1998). Tree species can be classified in ecological groups that create a specific set of environmental constraints. Likewise different AFS react differently to environmental constraints resulting in a specific understory for each tree species. Thick litter layers may physically hamper generative (e.g. Eriksson 1995) and vegetative (e.g. Sydes and Grime 1981a) propagation for some species like *A. nemorosa, Holcus mollis* L. and *P. trivialis* while other species like *H. non-scripta, L. galeobdolon* or *V. riviniana* will grow well in this environment giving them a competitive advantage (Sydes and Grime 1981a). *H. non-scripta*, an acid tolerant species with large seeds germinating in thick litter layers (Knight 1964) and which is taking advantage of the early spring to escape the dark shade, is well adopted to situations in for example *F. sylvatica* forests (Blackman and Rutter 1954). This might mean that solving the colonisation bottleneck of different AFS consequently needs the use of different tree species. To better understand the complex tree species-understory relation affecting the colonisation of post-agricultural forest, further research on litter and light regime of both the generative and vegetative colonisation of these plants would be of interest.

Colonisation of AFS is a limiting factor for the success of ecological restoration of forest habitat. Seed availability and subsequent germination and establishment are bottlenecks for colonisation (Ehrlén and Eriksson 2000; Verheyen and Hermy 2004). Based on our results, I conclude that tree species can have an important impact on this colonisation success because they strongly influence the germination and establishment of AFS. This tree species effect is especially important since it is one of the few factors (e.g. light management, De Keersmaeker et al. 2010) which can be controlled through management. When selecting tree species for post-agricultural forests one must be aware of the rapid soil acidification potential of species such as *A. glutinosa* and *F. sylvatica* (e.g. Neirynck et al. 2000). On acidification sensitive soils this effect is already present a few decades after afforestation of more or less neutral soils and can strongly affect the colonisation potential for AFS. When aiming to restore species rich forest habitats on these soils, especially when goal species are acid sensitive, I suggest that trees with a good decomposable litter like *Populus spp.* and *F. excelsior* are at least intermixed in afforestation.

5

Effects of canopy closure scenarios on the colonisation of forest herbs: first results

Abstract

Tree species effects on the herb layer are mainly explained by soil acidification and light availability. In contrast to soil acidification, the impact of light transmittance on the herb layer is not studied in great detail. Especially, the canopy closure of different tree or shrub species might have a much bigger impact on the understory than the summer light transmittance. Here the first results are presented of a sowing experiment under different canopy closure scenarios, representing an early, middle and late leafing out shrub layer, besides a control. Furthermore, the competitive herb layer was removed in half of the plots. It is hypothesized that late emerging or flowering and evergreen AFS are more dependent on shrub species that leaf out late and thus have lower survival under shrub species that leaf out early. In contrast, early emerging AFS might benefit from early shade which can suppress competitors more easily. Furthermore, an effect on the preferred scenario of the seed weight might be expected. Only for late flowering *S. holostea*, the recruitment in the middle scenario was found to be higher than in the late scenario and this only in the plots with competition and only for the second year after sowing. Furthermore, *A. nemorosa*, *P. elatior*, *S. holostea* and *M. effusum* have a higher recruitment under one of the three canopy closure scenarios compared to the control plots. The preferred canopy closure coincides with their flower and leafing phenology of the species. *V. riviniana, H. non-scripta* and *L. galeobdolon* were not affected by canopy closure. The recruitment of two hemicryptophytes, *V. riviniana* and *M. effusum,* improved by removal of the competitive herb layer. It is concluded that recruitment during the first two years is unlikely effected by differences in the canopy closure of the shrub layer. It is expect that canopy closure might be more relevant for later life stages when competitive exclusion becomes more important.

5.1 Introduction

Tree species effects on the understory species assemblage are well studied in temperate forest and it is concluded that this tree species effect can be mainly explained by the litter quality induced soil acidification and light transmittance of the overstory (Barbier et al. 2008). In many studies, the light transmittance is measured at a single time interval in the summer to characterise differences between tree species (e.g. Augusto et al. 2003; Aubert et al. 2004; van Oijen et al. 2005; chapter 2 and 3). Understory light levels in temperate forests are, however, highest in spring at April or May (e.g. Komiyama et al. 2001a; Augspurger et al. 2005). Many AFS have adopted there phenology to this pattern and therefore it is likely that the leaf flush of different tree and shrub species has a much bigger impact on the understory than the summer light transmittance. The timing of canopy closure of different trees is found to differ up to six weeks (Komiyama et al. 2001a; Augspurger and Bartlett 2003; Richardson and O'Keefe 2009; Dreiss and Volin 2013).

The spring phenology of tree saplings is a few days or weeks earlier than of canopy trees of the same species. However, for some tree species a reverse pattern is found (Augspurger and Bartlett 2003; Augspurger et al. 2005; Richardson and O'Keefe 2009). In autumn, phenological differences between saplings and canopy individuals are absent or very small. Also herb layer species appear to have adopted a, so called, phenological escape strategy in spring but rarely in autumn, taking advantage of the high-light period in spring before canopy development (Rothstein and Zak 2001). The phenological escape of understory herbs and saplings brought the majority of their total annual carbon gain (Seiwa 1998; Rothstein and Zak 2001; Augspurger et al. 2005).

The spring escape period differs between herb layer species (Landhäusser et al. 1997; Rothstein and Zak 2001; Kudo et al. 2008). Kudo et al. (2008) grouped herb layer species according their phenology in (1) spring bloomers, flowering and fruiting before the completion of canopy closure; (2) early summer bloomers, flowering during the progress of canopy closure and fruiting after canopy closure; and (3) late-summer bloomers, flowering and fruiting after canopy closure. Rothstein and Zak (2001) studied three phenologically different herb layer species. Leaves of the spring ephemeral showed characteristics of sun-plants, the summer-green species adopted its chemical, photosynthetic and structural leaf characteristics progressively during canopy closure, while the semi-evergreen showed leaf adaptation in the autumn. Graves (1990) found similar adaptations during the canopy closure for *M. perennis* and *G. urbanum*.

Differences in leaf phenology between canopy species have been found to influence the understory composition (Komiyama et al. 2001a; Dreiss and Volin 2013). The latter authors compared five tree species and found higher invasive species establishment under trees with later bud burst, leaf flush and total seasonal amount of light transmittance. A transplant experiment of an herb layer species under tree species with different canopy closure showed higher growth under late leafing tree species (Komiyama et al. 2001b).

Some effects of overstory phenology have also been found to effect germination and recruitment of trees. Large-seeded species unfold their large leaves in a short period after germination avoiding shade stress of early canopy closure resulting in higher survival (Seiwa and Kikuzawa 1996). In contrast, small-seeded species have a longer duration of leaf emergence, shorter leaf longevity, and rapid leaf turnover under different canopy conditions resulting in lower survival (Seiwa and Kikuzawa 1996).

As competitors do not show a spring escape strategy, they can probably be suppressed by an early leave flush and low summer light levels (see previous chapters). Many AFS, likely, utilize the high spring light level for their carbon gain, while summer-green AFS might depend more on a late canopy closure. Consequently, an optimal canopy closure scenario might suppress competitors but stimulate the recovery of AFS in post-agricultural forests. As, little research has been performed on phenological effects of different canopy species on the understory assemblage, especially for European species.

Previous chapters learned that moderately acidifying tree species are interesting for the restoration of a typical herb layer in post-agricultural forests. Temporary light gaps increase the establishment of AFS, while high shade levels promote the vegetative expansion of AFS (De Keersmaeker et al. 2011b; chapter 3). Consequently, it is interesting to have a moderately acidifying tree species with high light transmittance (e.g. *Populus* spp., *Salix* spp., *P. avium, F. excelsior*) with a coppiced shrub layer. Selection of a shrub species with an optimal canopy closure scenario could, furthermore, improve the herb layer recovery. Therefore, a long term experiment was set up in 6 stands (2 forests), each with a moderately acidifying tree species with high light transmittance. In these stands AFS with various spring escape phenologies were sown under four different canopy closure scenarios in plots with and without the competition of the original herb layer. Here, the results of the first two years are presented.

It is hypothesized that spring ephemeral species have higher recruitment under the early canopy closure scenario than summer-green and semi-evergreen species which depend on late canopy closure (cf. Rothstein and Zak 2001; Kudo et al. 2008; Dreiss and Volin 2013), large-seeded species might germinate earlier, resulting in higher survival under the early canopy closure scenario (cf. Seiwa and Kikuzawa 1996). Furthermore, an effect of herb layer removal on the recruitment might be expected (cf. Eriksson 1995; Verheyen and Hermy 2004; Baeten et al. 2009c). It is also expected that cover of competitors will decrease with earlier canopy closure resulting in an indirect effect of the canopy closure scenarios.

5.2 Material & Methods

5.2.1 Experimental set up

I selected 6 stands in 2 different forests (i.e. Deinze: 51.00° N, 3.54° E and Grimminge: 50.78° N, 3.93° E). Both forests are post-agricultural forest planted on loamy soil sensitive for acidification. All stands have a homogenous overstory of a moderately acidifying tree species with high light transmittance to have a similar set up (Table 5.1). The stands in Grimminge are planted to collect flowers and seeds for breeding and crossing experiments, the *Prunus* and *Ulmus* stands are crafted and consequently grow less tall and have higher light transmittances than in natural circumstances. In the stands of Grimminge the shrub layer was absent due to mowing and shrub layer was scarce in Deinze. In each stand, 4 plots of 3.5x3.5 m with similar vegetation and light level were selected. On one half of each plot, the natural herb layer was removed by weeding in June 2011. During 2011 and 2012 sprouts of the natural herb layer in the competition free parts were clipped. In each plot, 28 subplots of 25x25 cm were positioned, half of them in the competition free part.

Seeds of seven AFS (Table 5.2) were collected in the direct surrounding of Grimminge. Selected species have a different phenology and seed weight but comparable shade tolerance (Table 3.3) so that differential reactions upon the canopy closure scenarios might be expected. Ripe seeds were collected in 2011 or 2012. Seeds were counted, weighted and sown in July and early August of the same year in two random subplots in each competition free and competition part of each plot (96 subplots for each species). Seeds were stored at a dry and dark location before sowing, except seeds of *A. nemorosa* which was sown in May within 2 days after collecting as it loses its germination ability quickly when stored (chapter 4).

Table 5.1 Overview of the stands with stand and soil characteristics. Stand characteristics measured in November 2013.

Forest, tree species	Planting date	Stem density (ha^{-1})	Basal area ($m^2.ha^{-1}$)	Mean height (m)	L (%)	Silt (%)	pH-H_2O	C/N	Kj-N	C	P	Ca
									(mg.kg^{-1})			
Deinze, *Fraxinus excelsior*	1996	1309	24.7	16.0	9	49.6	5.07	15.9	2586	3.99	460	2905
Deinze, *Prunus avium*	1996	971	27.0	14.5	12	49.3	4.77	14.2	2936	4.12	440	2309
Grimminge, *Populus trichocarpa*	1974	374	46.4	32.7	10	55.4	5.55	16.5	2174	3.59	390	2081
Grimminge, *Prunus avium*	1986	861	23.3	13.4	8	50.6	5.89	15.0	1874	2.79	588	1772
Grimminge, *Salix alba, S. fragilis* and *S. x rubens*	1997	764	35.2	21.6	13	62.6	6.28	13.0	5244	6.79	1293	6183
Grimminge, *Ulmus x hollandica*	±1975	376	39.5	16.7	5	51.2	5.57	16.1	1836	3.00	420	2078

L: Summer light transmittance measured with densiometer (2010)

On three of the four plots, a shade treatment was applied with the same shade level in the summer but different application timing in the spring. The fourth plot was kept as control and did not receive any shade nets. A wooden construction of 0.5 m high was placed above each plot. As I expected the herb layer to grow taller in the control plots, the constructions of the control plot were 2 m high. A chicken wire was attached on top and around each construction to attach the shade nets and to prevent birds to remove seeds and seedlings. Shade nets were placed on top and along the sides of the constructions (Table 5.3). To select realistic shade treatments, light measurements were performed in the stands in the summer of 2010 (Table 5.1). The length of the canopy closing (2 months) and summer shade level (92% + canopy shade) were based on previous light measurements in dark stands (Verstraeten et al. 2004; Van der Aa unpubl. data; chapter 3). The different variants of the canopy closing scenarios were based on literature (see 5.1 Introduction) and field observations of early, middle and late emerging trees and shrubs (respectively *Prunus padus, C. avellana* and *F. excelsior*). Shade treatment started in spring 2012 and was repeated yearly.

5.2.2 Measurements and statistics

Soil samples of the top 0-5 cm mineral soil were collected in June 2011 in both the parts with and without competition as a mixture of eight subsamples. Soil samples were analysed for soil texture (Vandecasteele et al. 2002; chapter 2), pH-H_2O (glass electrode), Kjeldahl Nitrogen (heat destruction with H_2SO_4 and K_2SO_4/$CuSO_4$ catalyst, analysed by titration in H_3BO_3), carbon (as 55% of the loss on ignition between 105 and 550 °C), P fractions (De Schrijver et al. 2012b) and base cations (NH^{4+}-acetate-EDTA extraction by atomic absorption spectrophotometry).

In May 2012, a vegetation relevé was made of the part with competition in each plot by recording the cover of all herb layer species according to the Londo scale (Londo 1976). Furthermore, the total covers of the herb and moss layer were estimated.

The number of seedlings in each subplot was counted in May, July and September 2012 and May 2013. For 2012, the percentage of seedling recruitment was calculated based on the highest number.

Table 5.2 Selected species with their shade tolerance (75% mL range of Cornelis et al. 2009, see Table 3.3), flower phenology and life form (Hermy 1985; Lambinon et al. 1998; Weeda et al. 1999), number and mean weight of seeds sown and year of seed collecting and sowing. He: hemicryptophytes, Ch: chamaephytes and Ge: geophytes. Species ordered by flowering phenology.

Species	Shade tolerance	Flower phenology	Life form	Nr of seeds /subplot	Mean (±s.e.) weight (mg)	Year
Viola riviniana	4.6-5.8	Very early	Semi-evergreen He	100	132 (±0.4)	2011
Anemone nemorosa	4.6-5.7	Very early	Spring ephemeral Ge	100	572 (±3.7)	2012
Primula elatior	4.5-5.9	Early	Summer-green He	100	54 (±0.3)	2011
Hyacinthoides non-scripta	4.7-5.7	Early	Spring ephemeral Ge	100	513 (±1.7)	2011
Stellaria holostea	4.8-5.8	Late	Evergreen Ch	100	90 (±0.6)	2012
Lamium galeobdolon	4.4-5.7	Late	Evergreen Ch	50	85 (±1.0)	2011
Milium effusum	4.5-5.6	Very late	Evergreen He	100	124 (±0.6)	2011

Table 5.3 Four shading scenarios with the dates of application of the different shade nets

Scenario and species based upon	Percentage shade produced by applied shade nets						
	35%	58%	81%	92%	58%	35%	0%
Early: *P. padus*	11/03	01/04	22/04	13/05	30/09	21/10	11/11
Middle: *C. avellana*	01/04	22/04	13/05	03/06	30/09	21/10	11/11
Late: *F. excelsior*	22/04	13/05	03/06	24/06	30/09	21/10	11/11
Control	Year round no shade nets						

Generalised linear mixed models (GLMM) were built to explain the recruitment of each species in each year by the different canopy closure scenarios (early/middle/late/control) and competition (with/free). The random effect included stand, as there was less variation in the recruitment between stands for the competition free subplots than for the subplots with competition, the random effect of stand was split up by the two levels of competition. Finally, the individual subplot was included in the random effect part to prevent overdispersion. Multiple comparisons were performed with Tukey test by a p-value of 5%. The structure of the model is:

Recruitment ~ 1 + Shade*Competition + (Competition|Stand) + (1|subplot)

Statistics were performed in R 3.0.1 with library lme4 (R Development Core Team 2009; Bates et al. 2012).

5.3 Results

All species germinated, mean recruitment is between 1.9% for *S. holostea* and 16.2% for *H. non-scripta* in the first year and between 1.7% for *L. galeobdolon* and 11.6% for *H. non-scripta* in the second year. The difference in recruitment between species is not related to seed weight (results not sown).

The general results of the GLMM models are given in Table 5.4. For half of the species the variance between stands is about double as high in the subplots with competition compared to subplots free from competition. For the other species the variance is similar between competition and competition free subplots. This is logically explained by the fact that the stands differ in herb layer and recruitment partly depends on these differences. Results of Tukey test are represented in Figure 5.1. Only for *M. effusum* and *V. riviniana*, the recruitment is higher in the competition free plots compared to the plots with competition and only in the second year.

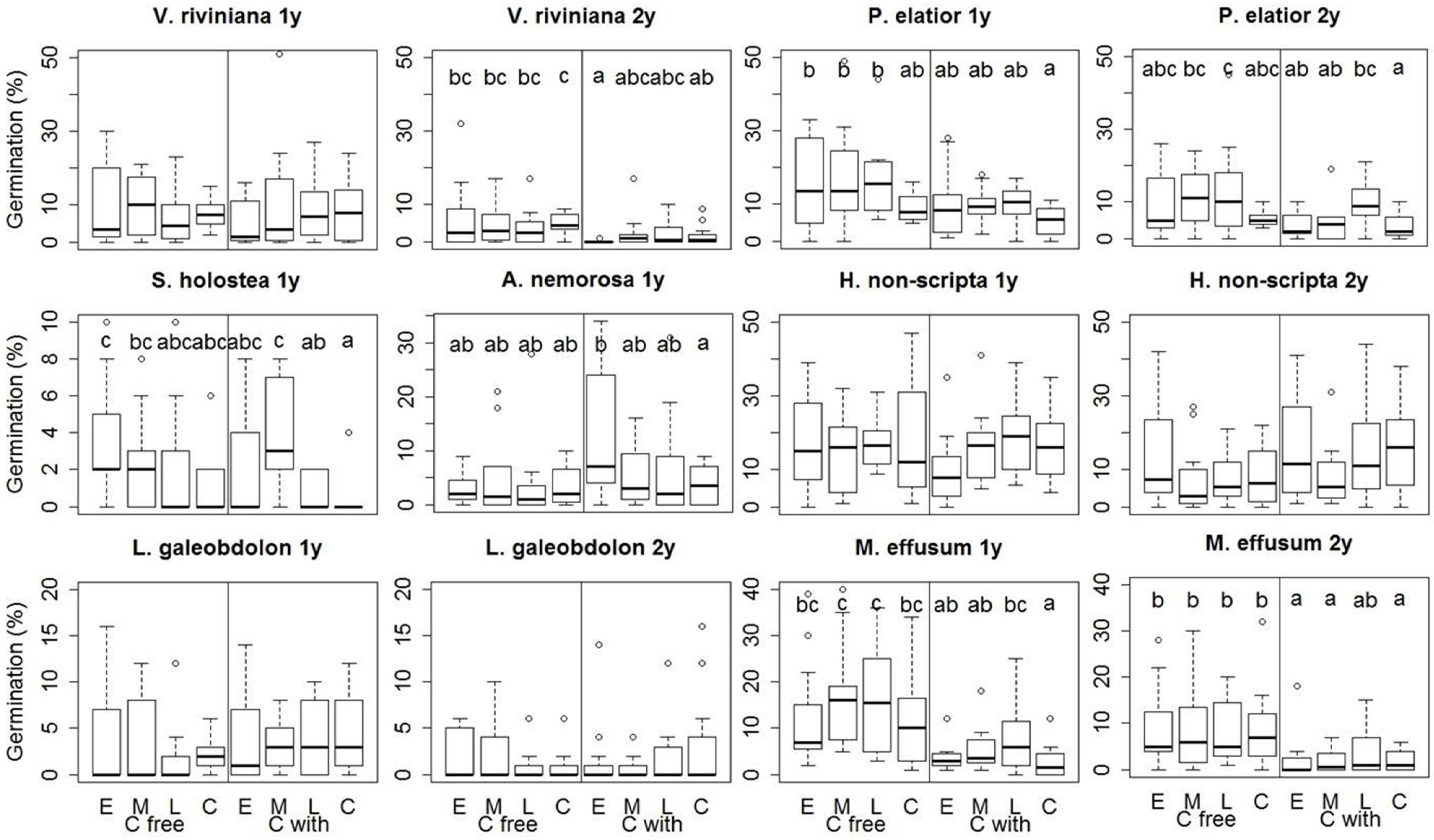


Figure 5.1 Boxplots of the different AFS and years separated for subplots with competition (C with) and free from competition (C free) and separated for the different shading scenarios (E: Early, M: Middle, L: Late and C: Control). Significant differences at $p<0.05$ with Tukey test are represented by different letters above the boxplot.

Table 5.4 P-values of GLMM explaining the recruitment of AFS by interaction of light transmittance and competition and standard deviation of random intercept for subplots with competition (C with) and free from competition (C free).

Species	Year	Li*Competition	1|Stand (C free)	1|Stand (C with)
		p	s.d.	s.d.
Viola riviniana	1 yr	0.227	0.8	1.3
	2 yr	0.029*	0.8	2.0
Anemone nemorosa[a]	1 yr	0.036*	0.8	1.8
Primula elatior	1 yr	0.901	0.6	0.7
	2 yr	0.324	0.7	1.0
Hyacinthoides non-scripta	1 yr	0.218	0.4	0.4
	2 yr	0.857	0.6	0.6
Stellaria holostea[a]	1 yr	0.020*	0.8	1.5
Lamium galeobdolon	1 yr	0.383	0.9	0.7
	2 yr	0.146	2.5	1.3
Milium effusum	1 yr	0.569	0.7	0.6
	2 yr	0.415	0.7	1.0

*: $p < 0.05$; [a]: species sown in 2012 instead of 2011

In the competition free subplots, no significant differences are present between the different canopy closure scenarios. In the subplots with competition, only five significant differences are found between shading scenarios. In four cases, the recruitment is higher in one of the shade scenarios compared to the control scenario. The spring ephemeral *A. nemorosa* had a higher recruitment in the early canopy closure, early flowering summer-green *P. elatior* preferred the late canopy closure, late flowering evergreen *S. holostea* preferred the middle canopy closure and very late flowering and evergreen *M. effusum* preferred the late canopy closure. Only for *S. holostea*, the recruitment in the middle scenario is higher than in the late scenario.

5.4 Discussion

The first results of this experiment confirm the fact that recruitment can be favoured by removing the competitive herb layer. However, the current results are inconclusive for the effect of the canopy closure. For four out of seven species the recruitment was higher in one of the shading scenarios compared to the control.

Temporarily removing the competitive herb layer often improves germination and recruitment (e.g. Eriksson 1995; Verheyen and Hermy 2004; De Keersmaeker et al. 2011b) but results might depend on life form and site conditions (Baeten et al. 2009c). Also in our experiment, (semi-)evergreen hemicryptophytes (*M. effusum* and *V. riviniana*) benefitted from removing competition while vernal geophytes were not affected.

For the recruitment of four species there is a combined effect of the presence of summer shade together with one of the three canopy closure scenarios. The presence of summer shade gives an indirect effect by supressing the competitors while the preferred canopy closure scenario coincides with the flower and leaf phenology of the AFS. The spring ephemeral species shows a preference for an early canopy closure, while summer- and evergreen species were found to germinate better under the middle or late canopy closure.

Only for the evergreen *S. holostea*, the recruitment in the middle scenario is higher than in the late scenario. As this result is only significant in the plots with competition, the survival might be limited by the stronger growth of the herb layer in the late scenario.

It is concluded that recruitment during the first two years is unlikely effected by differences in the canopy closure of the shrub layer. It is expected that the survival of the AFS in the subsequent years might depend more on the canopy closure scenario as competitive exclusion becomes more important in later life stages (e.g. Baeten et al. 2009c). Therefore, the experiment should be followed up for some more years.

6

Mechanisms behind and interactions between soil acidity and light as tree species effects on forest herbs

After: Thomaes, A., De Keersmaeker, L., De Schrijver, A., Baeten, L., Vandekerkhove, K., Verstraeten, G. & Verheyen, K. 2013. Can soil acidity and light help to explain tree species effects on forest herb layer performance in post-agricultural forests? Plant and Soil, 373: 183-199

Abstract

Tree species affect herb layer species through their effects on soil quality and light regime but their relative importance and interactions are insufficiently known. Therefore, a pot experiment with soil taken from stands planted with tree species with contrasting effects on soil acidification, two light regimes and six forest perennials was set up. The survival or growth of *M. perennis, L. galeobdolon, A. nemorosa* and *P. elatior* was lower in the acid *Alnus* soils than in the less acid *Fraxinus* soils. By contrast, the acid tolerant *C. majalis* and *D. dilatata* were barely affected by tree species. Light conditions had less impact than soil chemistry and did not compensate for unfavourable soil conditions. Ca and P concentrations increased in plants grown in *Fraxinus* soils. The Mg and Al shoot/root ratios of respectively one and two of the acid tolerant species was elevated in the most acidified soil. It is concluded that tree species effects on forest perennials are mainly explained by increased Al concentrations under acidifying species. Changed plant concentrations and allocation are likely associated to Al antagonism. I found no light compensation for the soil effect on the studied species. However, light alters the plant nutrient concentrations and allocation which may suggest an indirect effect.

6.1 Introduction

Soil acidification by tree species is caused by (1) the time delay between the uptake of base cations in exchange for H^+ and the release of these base cations by the decomposition of the organic matter (Nilsson et al. 1982), (2) the production of organic acids by slowly decomposing litter (Devries and Breeuwsma 1986) and, (3) N_2-fixation by symbiotic bacteria of actinorhizal tree species (Van Miegroet and Cole 1984). This effect is even more profound in post-agricultural forests since these soils were regularly limed and often have a near neutral soil at the time of afforestation. In this case tree species have a rapid and diverging effect on soil pH (De Schrijver et al. 2012a; Sauer et al. 2012). The Ca concentration in the litter is one of the main drivers of tree species effects on the soil chemistry (Reich et al. 2005). Tree species litter poor in Ca will cause a decrease of the soil pH, exchangeable Ca and base saturation (Reich et al. 2005; De Schrijver et al. 2012a). Soil acidification and increasing Al availability in the soil solution result in decreasing earthworm abundance and diversity (Muys and Granval 1997; Reich et al. 2005; Edwards et al. 2009). Because earthworms are keystone species for litter decomposition, decreasing earthworm populations will lead to further litter accumulation and soil acidification (Reich et al. 2005; Edwards et al. 2009). Soil acidification and the associated increase in Al availability, can also lead to Al toxicity and nutrient imbalances in plants (Cronan and Grigal 1995; Weber-Blaschke et al. 2002). Acid intolerant tree species are found to suffer from Al accumulation within the root tissues and Ca, Mg and/or P deficiency due to Al antagonism (Weber-Blaschke et al. 2002). Further, acid intolerant species are also more Ca and Mg depending than other species (e.g. Falkengren-Grerup et al. 1995b; Lucash et al. 2012). Plant species that are adapted to grow in more acidic soils can show stress avoidance or tolerance strategies.

N_2-fixing tree species can create elevated soil N concentrations (Van Miegroet and Cole 1984). Elevated N concentrations were found to improve biomass production of only few forest herbs and half of the forest grasses studied by Falkengren-Grerup (1998), while very high N concentrations negatively affected most species. Summarizing, tree species can alter the H^+ activity, available base cations and Al concentration, P availability and N concentration in soils which might in turn affect the herb layer composition.

Another important effect of tree species on the herb layer is the varying light regime that they create (Barbier et al. 2008). Since understory light availability is correlated with temperature and humidity regimes at the forest floor it should be treated as a synthetic factor grouping all these effects (Barbier et al. 2008). Many forest forbs show either shade avoidance, by adjusting their phenology to match the higher light availability in the early spring, or shade tolerance by developing leaves with a low light compensation point

(Rothstein and Zak 2001). This might explain the slow and limited reaction in growth and cover on increased light levels (Thomas et al. 1999; Lindh 2008). Flowering of AFS, however, increases rapidly in response to increased light levels (Lindh 2008; Baeten et al. 2010).

Several long-term vegetation studies have found that altered light conditions and soil acidity, as a consequence of acid deposition and change in management, and canopy composition, has strongly altered the herb layer in the past decades but untangling these causes is difficult in field studies (Taverna et al. 2005; Van Calster et al. 2008; Baeten et al. 2009a). Moreover, some research has proposed a differential reaction of herb layer species on soil fertility or acidification depending on the light availability or vice versa. A pot experiment by Moir (1966) showed that the biomass production of the light demanding grass, *Festuca idahoensis* Elmer, was reduced by soil acidification, but this was more explicit in a shady environment than under full light exposure. A similar interaction has more recently been shown for seedlings of several tree species (e.g. Grubb et al. 1996; Bigelow and Canham 2002; Portsmuth and Niinemets 2007; Palow and Oberbauer 2009). Portsmuth and Niinemets (2007), for instance, demonstrated that the improving growth of tree species in response to increasing light was enlarged by nutrient addition and that this adaptation was greater for shade-tolerant trees.

In sum, tree species may have a profound impact on the colonisation success of herb layer species in forests established on arable land through the differences in soil chemistry and forest floor light regime they create. The colonisation rates of many of these herb layer species (the so called ancient forest plant species, Hermy et al. 1999) is known to be low, and this has been explained as a combination of dispersal and environmental limitation (Verheyen et al. 2003a; Hermy and Verheyen 2007; Baeten et al. 2009b). Tree species selection can reduce or improve this hampered colonisation by their impact on the recruitment and survival success of herb layer species (chapter 2 and 4).

Previous chapters revealed a tree species effect on the colonisation of AFS through their impact on soil acidity and light regime. In this chapter, I want to investigate the relative importance and interaction of the soil acidification and light regime of different tree on the survival, performance and plant nutrient status of the herb layer species. Therefore, I have set up an experiment with six forest perennials planted in soil collected under two tree species, *A. glutinosa* and *F. excelsior/americana*, which have a contrasting influence on the development of post-agricultural forest soils. Soil was collected in a young and an older afforestation of both tree species. The plants were treated with two different levels of light. I hypothesize 1) a negative effect of soil acidification on herb layer species performance and nutrient status, but 2) a positive effect of P and N availability on herb layer species performance and nutrient status, and 3) possible interactive effects between light and soil conditions. Similar to Moir (1966), I expect a more strongly reduced performance in acidified soils under shaded conditions compared to light rich conditions. Furthermore, I hypothesize that 4) the responses differ considerably among herb layer species and these differences are related to their ecological optimum for soil acidity and light.

6.2 Material & Methods

6.2.1 Plots and soil collection

The soil used in the experiment came from two post-agricultural forests, Mortagne forest (see chapter 1) and Eeuwenhout (50.77° N, 2.77° E, 70 m a.s.l.), planted on well fertilised loam soil (Haplic Luvisols). According to all available historical maps, both forests have been continuously used as farmland from 1850 until the afforestation in 1972 (Mortagne forest) and 1990 (Eeuwenhout).

The soil was collected under two tree species with contrasting effects on soil characteristics, *A. glutinosa* and *F. excelsior/americana*. Both are pioneer tree species, the first one is able to grow in waterlogged soils while *Fraxinus spp.* prefers humid soils. But both species grow good when planted on well-drained soil as in both forests. *Fraxinus spp.* have a good decomposable litter maintaining high soil pH (Cote and Fyles 1994; De Schrijver et al. 2012a). The litter of *Alnus* has a moderate quality but a symbiosis with N_2-fixating bacteria (*Frankia alni*) is known to increase the soil N concentration and acidifies the soil (Van Miegroet and Cole 1984). In Eeuwenhout a block design (blocks of 9x9 m) of different tree species is available, here four blocks with *A. glutinosa* and four with *F. excelsior* were randomly selected for soil collection. In Mortagne forest soil was collected in two locations in two stands of each tree species (*A. glutinosa* or *F. americana*). *F. americana* has a similar litter quality as the native *F. excelsior* (De Schrijver et al. 2012a). In each of the 16 plots about 70 l of mineral top soil (0-10 cm) was collected in November 2008. The soil was sieved and roots, branches and leaves were removed. A subsample was used for chemical analyses.

Differences between tree species in litter accumulation can mechanically limit the performance of the perennials (Sydes and Grime 1981a). The litter effect was, however, not included in the experiment as it is difficult to manipulate in a pot experiment.

6.2.2 Forest herb species selection

Five AFS with varying tolerance for soil acidity and shade were selected form Table 3.3: *P. elatior*, *M. perennis*, *L. galeobdolon*, *A. nemorosa* and *C. majalis* (Table 6.1). In order to be able to perform plant analyses, it was important to include at least some species that produce enough biomass and survive in acid soils. Therefore, *C. majalis* was selected. Further, *D. dilatata* was added as a strongly acid tolerant species as there are no AFS that are strongly acid and shade tolerant and produces enough biomass. Ellenberg et al. (1992) and Hill et al. (1999) categorise *D. dilatata* as intermediate shade tolerant, L-value respectively 4 and 5, while Cornelis et al. (2009) found it mainly on locations with more light (mL: 5.8, 75% range: 5.1-6.5). Three of the species were retrieved from a commercial nursery specialised in wild plants and the other three were collected in the forest. Half of the species are geophytes, the others are hemicryptophytes or chamaephytes. All species, but the fern *D. dilatata*, are spring flowering.

6.2.3 Experimental set-up

Perennials were planted in plastic flowerpots (2.2 l) with soil from one of the 16 plots in November 2008. The original soil was washed off the roots and plants were weighted before planting. In total 480 flowerpots were filled in a full block design with five replications of each of the six species in soil of the 16 plots (four plots of each of the two tree species in each of the two forests).

Table 6.1 Plant origin, mean weight, acid and shade tolerance (Table 3.3) and plant performance indicators for each plant species.

	Plant origin	Mean (±s.e.) weight (g)	Life form	Acid tolerance	Shade tolerance	Vegetative performance				Generative performance		
						Nl	Lll	PlH	PlC	Np	Llp	Nf
P. elatior	Nursery	44.5±13.4	He	Intolerant	Intermediate	-	X	X	X	X	X	X
M. perennis	Aelmoeseneie f 50.98°N,3.88°E	3.7±1.8	Ge	Intolerant	Tolerant	X	X	X	X	X[b]	-	-
A. nemorosa	Nursery	1.7±0.4	Ge	Intermediate	Intermediate	X	X[a]	X	X	X	X	-
L. galeobdolon	Mortagne forest	1.7±1.0	Ch	Intermediate	Tolerant	X	X	-	X	-	-	X
C. majalis	Nursery	20.8±9.1	Ge	Tolerant	Intermediate	X	X	X	X	X	X	X
D. dilatata	Vossekot f 51.17°N,4.21°E	67.0±34.8	He	Tolerant	Intermediate[d]	X	X	X	X	X[c]	-	-

He: hemicryptophyte; Ge: geophyte; Ch: chamaephyte; Nl: Number of leaves; Lll: Length of longest leaf; PlH: Plant height; PlC: Plant cover estimated in cover classes by comparing plants with reference areas of cover classes indicated on a sheet of paper; Np: Number of pedicels; Llp: Length of longest pedicel; Nf: Number of flowers; X: measured for this species; -: not measured for this species; [a]: Length of blade; [b]: Number of flowering shoots, [c]: Sporulating leaves and [d]: based on Ellenberg et al. 1992 and Hill et al. 1999.

The plants were placed on a root cloth in an open nursery in Geraardsbergen (50.76° N, 3.88° E), bordered by a forest at the southwest and southeast. Additional shading was applied to simulate a dense canopy cover as a second tree species effect. An equal number of plants of each forest x tree species x herb species combination were assigned to the shaded and unshaded treatment (alternating 2/3 and 3/2 per plot). The plants in the shaded blocks were each covered with shade nets creating 60% light reduction from April 10 2009 and 2010 onwards. About three weeks later (April 30), the shade nets were doubled. In the second year, the shade net was quadrupled from June 1 because I evaluated that there was still too much light in the shade treatment. All nets were removed on October 22. This shade regime was based on data extracted from repeated hemispherical photographs taken in closed *F. sylvatica* stands (Van der Aa unpubl. data). Blocks without shade nets received only low levels of shade from the nearby forest and were covered with a bird net from April 8 to October 22, ensuring a more or less similar barrier for pollinators compared to the shaded regime. PAR Light measurements were performed before and after placing shade nets and on monthly intervals in each block and on a nearby open field with an LAI-2000 Plant Canopy Analyzer. In the blocks with light treatment the summer light transmittance amounted to 60 ± 7% and 67 ± 6% in the first and second year, respectively, and 22 ± 4% (year 1) and 9 ± 2% (year 2) for the blocks with a shade treatment (Figure 6.1).

6.2.4 Plant performance

To evaluate the vegetative and generative performance of the plants, four vegetative and three generative plant performance indicators were measured in April, May, June and August 2009 and 2010 (Table 6.1). The shoots of *L. galeobdolon* grew fast and had the tendency to root in nearby flowerpots. Therefore, shoots of this plant were clipped and reduced to two nodes in August 2009, the plant material was retained for chemical analyses. All plant species were completely harvested in 2010 at their maximal cover, i.e. at the beginning of June for *A. nemorosa*, *M. perennis* and *P. elatior* and early August for the other species. Above and belowground biomass were collected separately and dried during one week at 50° C to obtain the dry weight.

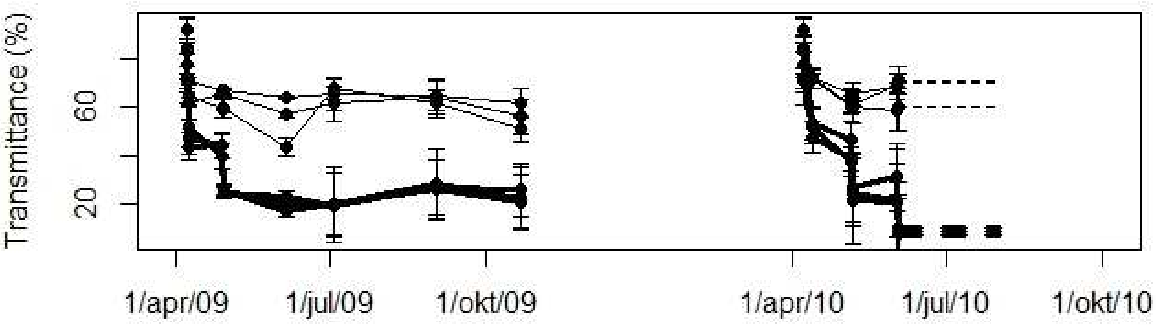


Figure 6.1 Mean (± s.e.) PAR light transmittance in the 6 blocks with shaded (bold line) and unshaded (thin line) treatment during the experiment. Dotted lines: estimates based on last measurement.

6.2.5 Soil and plant chemical analyses

Soil samples, collected at the beginning of the experiment, were analysed for their soil texture with laser diffraction (Vandecasteele et al. 2002; chapter 2), pH-H_2O and pH-KCl (glass electrode), bio-P (Bray and Kurtz 1945), tot-P (destruction with HCl and HNO_3 in a microwave oven, analysed with ICP), Kj-N (heat destruction with H_2SO_4 and K_2SO_4/$CuSO_4$ catalyst, analysed by titration in H_3BO_3), C (as 55% of the loss on ignition between 105 and 550° C), Ca, Mg, K and Al (NH^{4+}-acetate-EDTA extraction by atomic absorption spectrophotometry).

The plant material was analysed on P (Bray and Kurtz 1945), Kj-N (destruction with H_2SO_4 and K_2SO_4/$CuSO_4$ catalyst, analysed by titration in H_3BO_3), Ca, Mg, K and Al (microwave extraction with HNO_3 and H_2O_2, measured with ICP). For some plants insufficient biomass was available for chemical analyses. If a compound sample of different plants with the same treatment and plot had sufficient biomass, this joined sample was analysed. Two species that produced enough biomass (*D. dilatata*, *C. majalis*) were analysed separately for the below and above ground biomass to obtain the shoot/root ratio of the Al, Ca and Mg concentrations.

6.2.6 Statistics

First, the differences between the soil characteristics of the plots (n=16) was analysed with ANOVA, with tree species (TS: *Alnus/Fraxinus*) and forest (Eeuwenhout/Mortagne forest) as main effects.

Next, the survival of each species was analysed with generalised linear mixed models (GLMM) with a binomial family. Tree species, light (shaded/unshaded), forest and initial plant weight (last one as co-variable) were used as fixed effects, plot as random variable. Then a principal component analysis (PCA) was performed on the vegetative and generative performance data from the second year for each species separately (cf. Baeten et al. 2009b). The first PCA axes were used as compound performance indicator and used as response variable in LMM with the same structure as the GLMM model. When the PCA of the generative data showed a strong grouping into flowering and non-flowering plants (i.e. *A. nemorosa*, *L. galeobdolon* and *C. majalis*), further analyses were performed on the binary data (non-flowering/flowering) with GLMM instead. Finally, LMM's were build explaining the weight after/before ratio and the plant concentration of Al, Ca, Mg, K, Kj-N and P and ion Al/Ca and N/P ratios. Finally the shoot/root ratio of the concentration of Al, Ca and Mg was analysed (LMM) to see whether the allocation differed between the treatments.

All models are of the form:
Variable ~ 1 + tree species * light * forest * plant weight + (1|plot)

For all these models backwards selection was applied on the interaction terms using a p-value of 5%. Fixed effects were kept in the model since they represent principal treatment effects of the experimental design. For some species no plants survived under certain treatment combinations or insufficient plant material was available for chemical analyses. Hence, not all models could be applied to all species. All statistics was performed in R 2.14.1 with the lme4 library for GLMM, nlme for LMM and vegan for PCA (R Development Core Team 2009; Bates et al. 2012; Oksanen et al. 2013; Pinheiro et al. 2013).

6.3 Results

The soil of Mortagne forest has a bit more silt and less sand than the Eeuwenhout soil (Table 6.2). pH-H_2O and pH-KCl are nearly a full unit lower under *Alnus* than *Fraxinus* but also lower in the older Mortagne forest than at Eeuwenhout (Table 6.2). Consequently, the Al concentration is lower under *Fraxinus*, but there is a large difference between the concentrations in the *Alnus* plots of the two forests. Both Ca and Mg were higher in the soil under *Fraxinus* and were lower in Mortagne forest, while K was not significantly affected by tree species or forest. In Mortagne forest the soil of *Alnus* contained more N and C, while soil C/N ratio of *Alnus* was higher in both forests. The total P concentration was higher in the *Alnus* plots of Mortagne forest and in *Fraxinus* plots of the Eeuwenhout. Bio-P, however, was higher under *Alnus* in Mortagne forest but no difference was found at Eeuwenhout. The bio-availability of P (bio-P/tot-P) was higher in Mortagne forest and was especially high in the *Alnus* plots in Mortagne forest.

Table 6.2 Parameter estimates of ANOVA explaining soil characteristics with baseline set as TS (tree species) = *Alnus* and Forest= Eeuwenhout.

	df	Intercept	TS	Forest	TS x Forest
Sand (%)	12,1,1,1	29.55^{***}	-1.83NS	-12.55^{***}	1.65NS
Silt (%)	12,1,1,1	45.95^{***}	1.48NS	9.78^{***}	-2.28NS
Clay (%)	12,1,1,1	24.78^{***}	0.40NS	2.78NS	0.63NS
pH-H_2O	12,1,1,1	4.93^{***}	0.89^{***}	-0.85^{***}	0.28NS
pH-KCl	12,1,1,1	4.04^{***}	0.90^{***}	-0.80^{***}	0.21NS
Al (mg.kg^{-1})	12,1,1,1	142^{***}	-79	322^{***}	-269^{***}
Ca (mg.kg^{-1})	12,1,1,1	1338^{***}	628^{**}	-603^{**}	500NS
Mg (mg.kg^{-1})	12,1,1,1	131^{***}	61^{**}	-41^{*}	41NS
K (mg.kg^{-1})	12,1,1,1	205^{***}	-23NS	1NS	-18NS
Log(Kj-N*100) (%)	12,1,1,1	3.17^{***}	0.08NS	0.62^{***}	-0.76^{***}
Log(C*100) (%)	12,1,1,1	5.59^{***}	-0.05NS	0.65^{***}	-0.72^{***}
C/N ratio	12,1,1,1	11.17^{***}	-1.20^{*}	0.49NS	0.16NS
Bio-P (mg.kg^{-1})	12,1,1,1	28.58^{***}	9.88NS	39.10^{***}	-37.88^{***}
Tot-P (mg.kg^{-1})	12,1,1,1	560^{***}	113^{*}	146^{**}	-230^{**}
Sqrt(bio-P/Tot-P*100)	12,1,1,1	2.25^{***}	0.14NS	0.85^{***}	-0.65^{**}

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; NS: not significant.

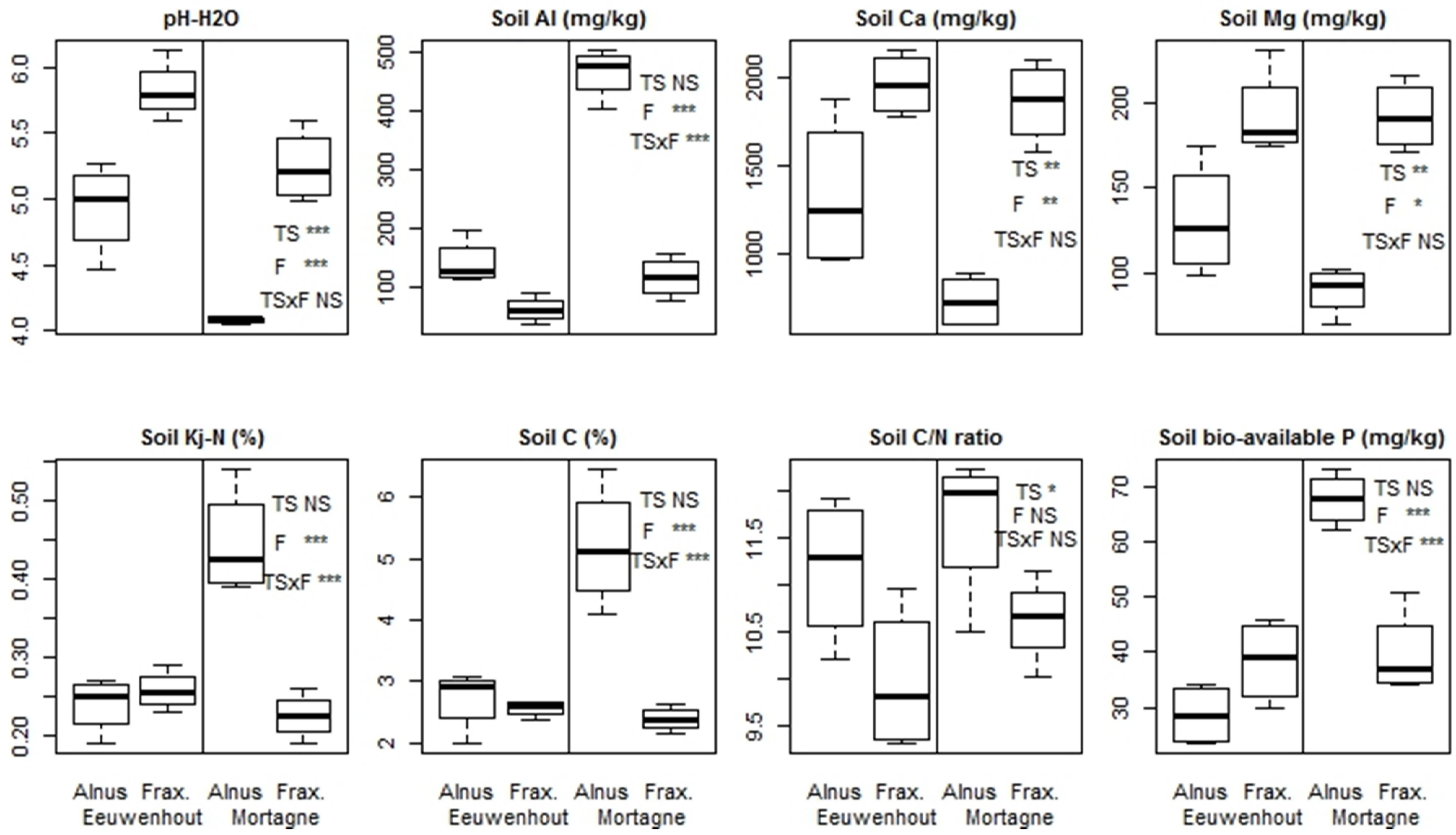


Figure 6.2 Boxplots of soil variables: pH-H_2O, Al, Ca, Mg, Kj-N, C, C/N ratio and bio-P concentration separated by treatments with significance of ANOVA test with TS: Tree species; F: Forest; NS: not significant; *: $p < 0.05$; **: $p < 0.01$ and ***: $p < 0.001$.

The survival of three species with no or intermediate tolerance for acidification (*M. perennis, L. galeobdolon* and *A. nemorosa*) was lower in soil of *Alnus* than in soil of *Fraxinus* (Figure 6.3). The survival of the acid tolerant *C. majalis* and *D. dilatata* was unaffected by tree species. All plants of the acid intolerant *P. elatior* survived the experiment (Table 6.3), but the weight after/before ratio of *P. elatior* revealed that plants had decreased in size except these in the soil of *Fraxinus* from Eeuwenhout. The individuals under *Alnus* from Mortagne were reduced to a fraction of their original weight. Furthermore, *P. elatior* has a higher vegetative and generative performance in soil of *Fraxinus* sampled at both forests. While the vegetative performance of *L. galeobdolon*, *A. nemorosa* and *C. majalis* in soil of *Fraxinus* was only higher in Mortagne forest. Also for *L. galeobdolon* and *A. nemorosa* the weight after/before ratio was higher in the soil of *Fraxinus* than in the soil of *Alnus* (Figure 6.4). In contrast to the vegetative response, flowering of acid tolerant *C. majalis* was enhanced by soil of *Alnus* in both forests. *M. perennis* and *A. nemorosa* had a higher survival in soil from Eeuwenhout and generative performance of *P. elatior* was enhanced in soil of this forest.

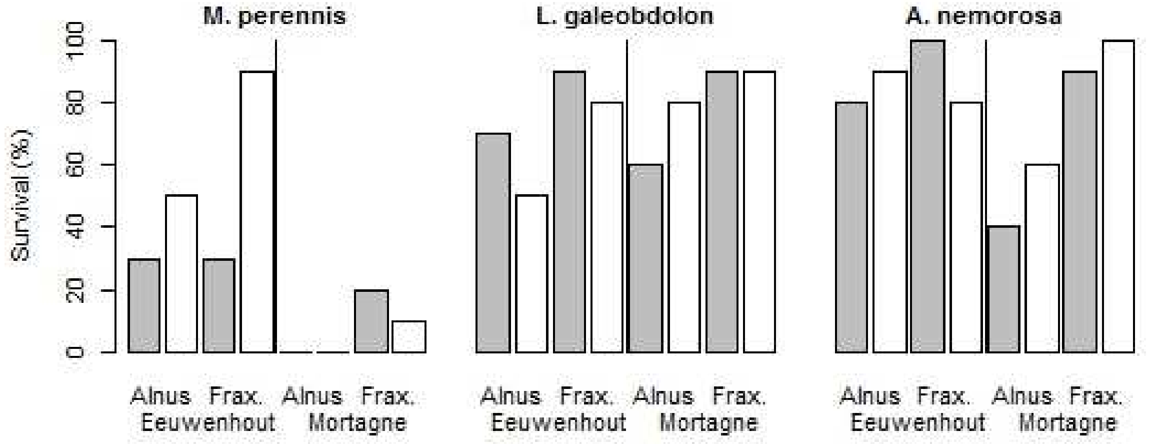


Figure 6.3 Bar plots of the survival (%) of *M. perennis*, *Lamium galeobdolon* and *Anemone nemorosa* separated by treatments. Grey bars: shaded plants and white bars: unshaded plants.

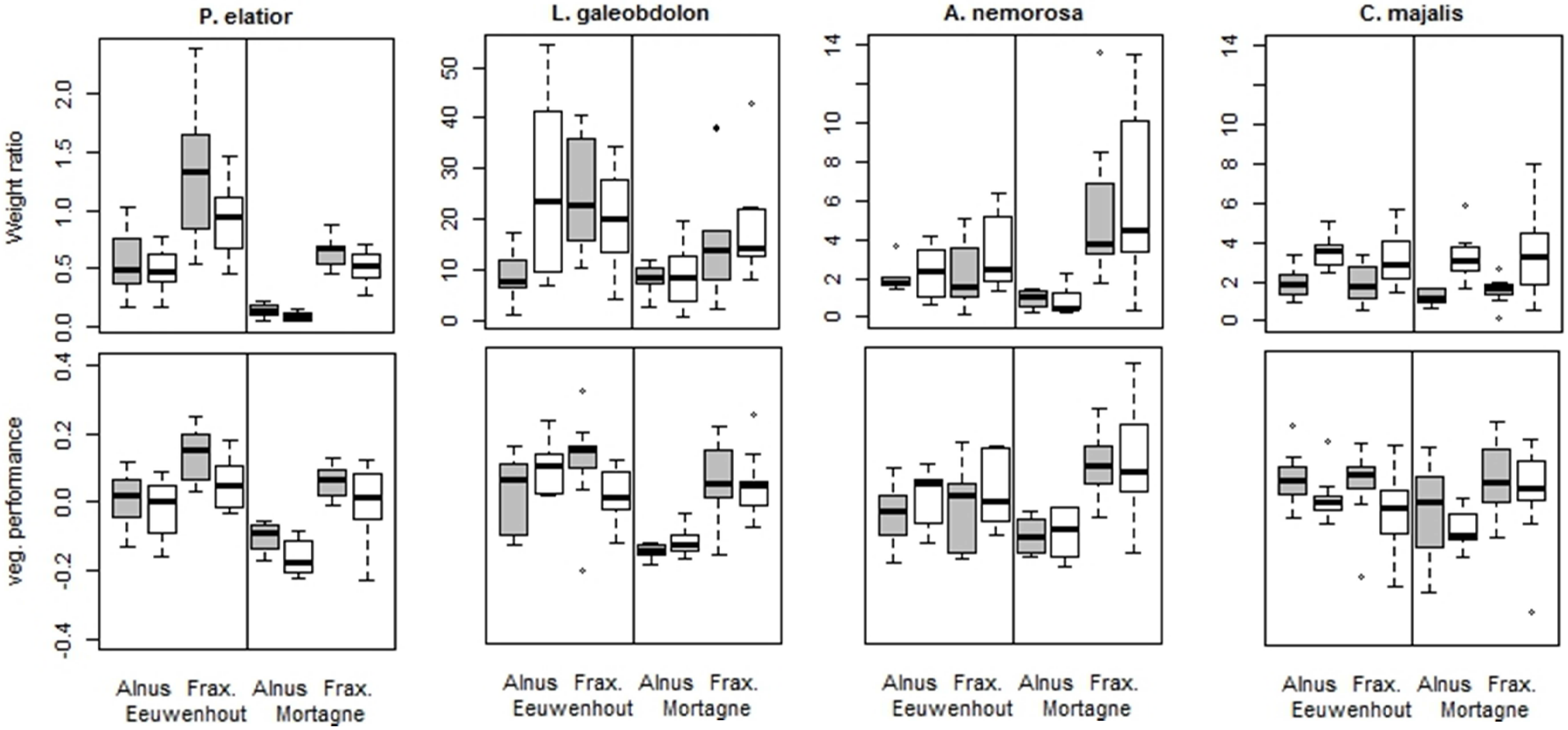


Figure 6.4 Boxplots of the weight after/before ratio and vegetative performance (first PCA axis on vegetation measurements, Table 6.1) of *P. elatior*, *L. galeobdolon*, *Anemone nemorosa* and *Convallaria majalis* separated by treatments. Grey boxes: shaded plants and white boxes: unshaded plants.

Table 6.3 Parameter estimates of GLMM’s and LMM’s explaining survival, vegetative and generative performance, binary generative data and weight after/before ratio with baseline set as TS (tree species)= *Alnus*, Light= shaded and Forest= Eeuwenhout. AFS are ordered by increasing acid tolerance and shade tolerance (see Table 6.1).

	df	Intercept	TS	Light	Forest	Initial weight	TS x Forest	Light x Weight
Survival								
P. elatior	62,14,14,14,62	0.21NS	-0.20NS	0.20NS	-0.18NS	0.003NS	-	-
M. perennis	62,14,14,14,62	-2.48**	1.60*	1.10NS	-2.87***	0.01NS	-	-
A. nemorosa	62,14,14,14,62,14	1.55NS	1.76*	8.90*	-1.43*	-0.001NS	-	-0.08*
L. galeobdolon	62,14,14,14,62	0.20NS	1.49*	-0.13NS	0.52NS	0.003NS	-	-
C. majalis	61,14,14,14,62	0.21NS	-0.20NS	0.20NS	-0.18NS	0.003NS	-	-
D. dilatata	62,14,14,14,62	0.58NS	-0.19NS	-0.20NS	-0.19NS	-0.03NS	-	-
Vegetative performance								
P. elatior	62,13,62,13,62	0.002NS	0.13***	-0.06***	-0.10**	0.0003NS	-	-
A. nemorosa	46,12,12,46,46,12	-0.15NS	0.02NS	0.04NS	-0.09NS	0.06NS	0.20*	-
L. galeobdolon	43,12,12,43,43,12	0.005NS	0.02NS	-0.0006NS	-0.17**	0.01NS	0.15*	-
C. majalis	59,12,59,12,59,12	-0.05NS	-0.02NS	-0.06**	-0.10**	0.01**	0.12*	-
D. dilatata	61,13,13,61,61	0.03NS	-0.03NS	0.12***	-0.03NS	-0.0008**	-	-
Generative performance								
P. elatior	62,13,13,62,62	-0.004NS	0.12***	-0.006NS	-0.09**	-0.0002NS	-	-
D. dilatata	61,13,13,61,61	-0.07NS	0.05NS	0.03NS	-0.005NS	0.0004NS	-	-
Binary generative data								
A. nemorosa	46,12,12,46,46	-3.36NS	1.04NS	0.70NS	0.89NS	0.46NS	-	-
L. galeobdolon	43,12,12,43,43	-19.10NS	17.70NS	0.10NS	-0.01NS	-1.02NS	-	-
C. majalis	59,12,59,12,59	1.23NS	-2.30*	-0.28NS	0.86NS	0.12NS	-	-

(table 6.3 continued)

Log (weight after/before ratio)								
P. elatior	61,12,12,61,12	-0.65***	0.83***	-0.30**	-1.53***	/	0.91**	-
A. nemorosa	45,12,12,45, 12	0.60*	0.04NS	0.14NS	-1.03*	/	1.76**	-
L. galeobdolon	48,13,13,48	2.31***	0.57*	0.18NS	-0.33NS	/	-	-
C. majalis	61,13,13,61	0.53***	-0.03NS	0.66***	-0.15NS	/	-	-
D. dilatata	62,13,13,62	0.38***	0.17NS	0.40***	-0.03NS	/	-	-

/: variable not included; -: variable excluded by model selection; NS: not significant; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Only few tree species effects were found on the plant concentrations of Al, Mg, K and N (Table 6.4). The plant Ca concentration in the acid tolerant *D. dilatata* and *C. majalis* was higher when planted in soil of *Fraxinus* (last one only in Mortagne forest). The Ca concentration in acid intolerant *P. elatior* was higher in soil of *Fraxinus* in Eeuwenhout but opposite in Mortagne forest. Finally, the Ca concentration in the intermediate acid tolerant *L. galeobdolon* is 15% higher in the *Fraxinus* soil, 28% higher in the intermediate acid tolerant *A. nemorosa* and 8% higher in the acid intolerant *M. perennis* (not significant). As there was no difference in Al concentration, the Al/Ca ratio did not differ between tree species either. The P concentration is significantly higher in three out of four species (*P. elatior, L. galeobdolon* and *D. dilatata*) in the soil of *Fraxinus* (Figure 6.5). The N/P ratio was lower in soil of *Fraxinus* for *L. galeobdolon* and *D. dilatata*, a consequence of the high P concentration.

A high light intensity reduced the plant concentrations of Ca, Mg, K, N and P in most of the cases, while the Al concentration increased in two plant species and decreased in a third one. There was a positive effect of light on the Al/Ca ratio for C. majalis and D. dilatata. Light negatively influenced the N/P ratio for all four species. For two species without compound samples (C. majalis and D. dilatata), it was possible to analyse the total content of Al, Ca, Mg, K, N and P as well (Table 6.5). These results were rather similar to the results on the concentration, except for the effect of light. Plants that received more light showed in general lower nutrient concentrations, but had higher total nutrient contents.

The ion shoot/root ratio was influenced more by the light treatment than by the tree species (Table 6.6). Al was stored mainly below ground by the acid tolerant species *C. majalis* and *D. dilatata*. Shaded plants generally had an elevated Al shoot/root ratio. The Al shoot/root ratio of both perennials was lower under *Fraxinus* than *Alnus* in Mortagne forest, while there is no difference between tree species in Eeuwenhout. The Ca shoot/root ratio was lower in the shaded plants, but did not differ between forest and tree species. The Mg concentrations were up to 2 times higher above than below ground and the shoot/root ratio in *C. majalis* was highest in shaded plants. In *D. dilatata* the shoot/root ratio was higher in plants in soil of *Alnus* of Mortagne forest than in other treatments. This indicates that more Mg is allocated from the roots to the leaves.

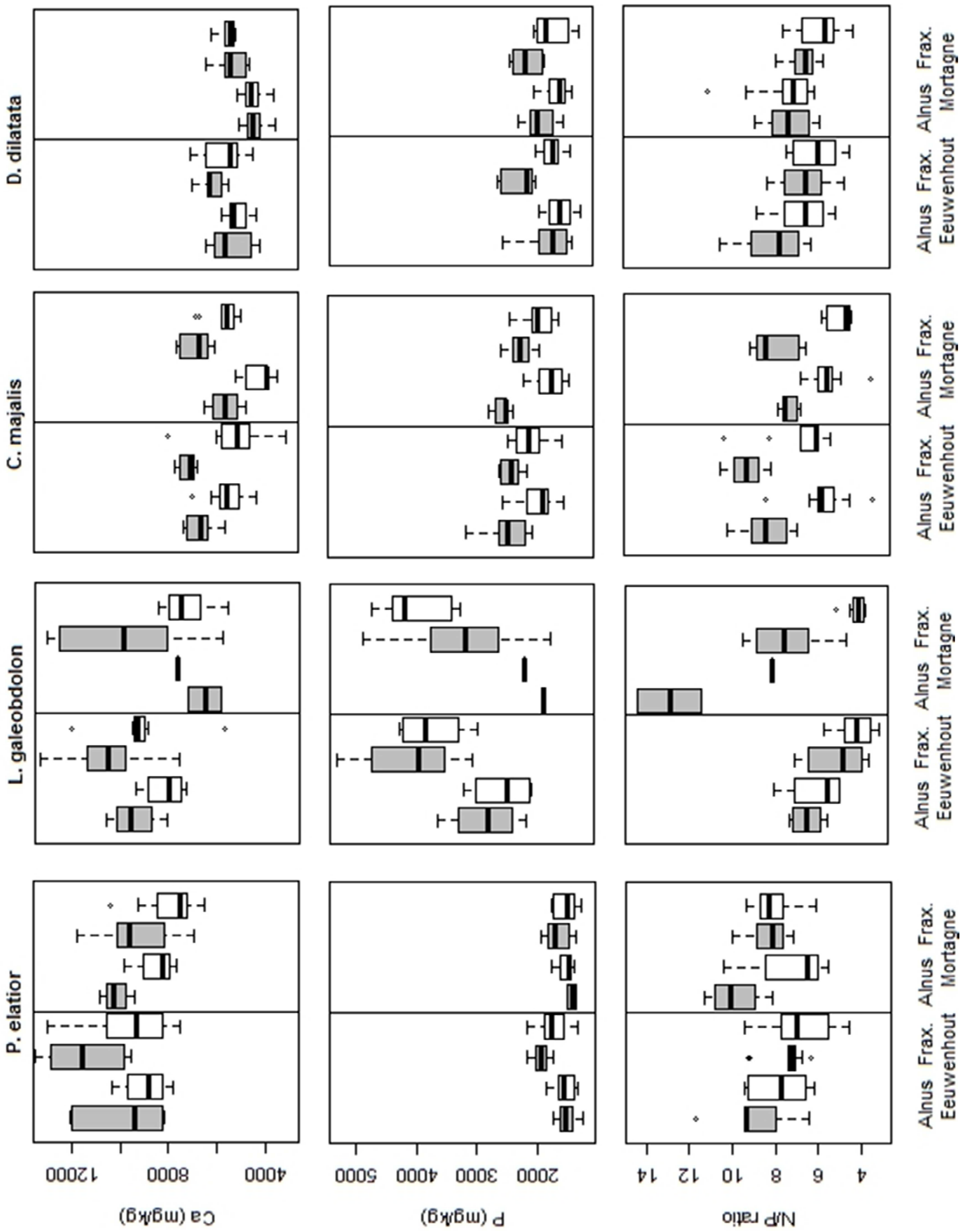


Figure 6.5 Boxplots of the plant Ca and P concentrations and N/P ratio in *Primula elatior*, *Lamium galeobdolon*, *Convallaria majalis* and *Dryopteris dilatata* separated by treatments. Grey boxes: shaded plants and white boxes: unshaded plants.

Table 6.4 Parameter estimates of LMM's explaining plant concentrations of Al, Ca, Mg, K, Kj-N, P, Al/Ca ratio and N/P ratio with baseline set as TS (tree species)= *Alnus*, Light= shaded and Forest= Eeuwenhout. AFS are ordered by increasing acid tolerance and shade tolerance (see Table 6.1).

	df	Intercept	TS	Light	Forest	TS x Forest	TS x Light	Light x Forest
Al (mg.kg^{-1})								
P. elatior	39,13,13,39	272***	-9NS	-66**	-54*	-	-	-
L. galeobdolon	29,9,9,29	138.8***	26.5NS	0.5NS	-48.3NS	-	-	-
C. majalis	51,13,13,51	889***	66NS	250**	230NS	-	-	-
D. dilatata	62,13,13,62	1770***	32NS	666***	503*	-	-	-
Ca (mg.kg^{-1})								
P. elatior	40,12,12,40,12	10198***	1080NS	-1471***	-68NS	-1865NS	-	-
L. galeobdolon	29,9,9,29	9426***	1410NS	-1660**	-1537NS	-	-	-
C. majalis	51,12,12,51,12	6801***	124NS	-1401***	-1179**	1246*	-	-
D. dilatata	61,13,13,61,61	5369***	811***	-342*	-874**	-	-	462*
Mg (mg.kg^{-1})								
P. elatior	40,13,13,40	2891***	223NS	-504***	-11NS	-	-	-
L. galeobdolon	28,9,9,28,28	3255***	1057NS	-1143**	-153NS	-	-	-90NS
C. majalis	51,13,13,51	1522***	32NS	-312***	148*	-	-	-
D. dilatata	62,12,12,62,12	2039***	220NS	-59NS	-152NS	335NS	-	-
K (mg.kg^{-1})								
P. elatior	40,12,12,40,12	24775***	-1933NS	-3854***	-9709**	10665*	-	-
L. galeobdolon	29,9,9,29	42775***	-2199NS	-8701***	-1238NS	-	-	-
C. majalis	51,13,13,51	14657***	-446NS	-5350***	-327NS	-	-	-
D. dilatata	62,12,12,62,12	9419***	-250NS	-1684***	-1198NS	1910NS	-	-

(table 6.4 continued)

Log(Kj-N*100) (%)								
P. elatior	40,13,13,40	4.907***	0.020NS	-0.160***	0.002NS	-	-	-
L. galeobdolon	32,9,9,32	5.262***	-0.004NS	-0.277***	0.152**	-	-	-
C. majalis	50,12,12,50,12	5.3***	0.2*	-0.6***	-0.1NS	-0.2NS	-	-
D. dilatata	61,13,13,61,61	4.91***	0.04NS	-0.20***	0.02NS	-	-0.17**	-
P (mg.kg^{-1})								
P. elatior	39,13,13,39,39	1527***	334**	101NS	-157NS	-	-253*	-
L. galeobdolon	28,9,9,28,28	2792***	1307**	-301NS	-910*	-	-	1061*
C. majalis	50,13,13,50,50	2524***	-160NS	-625***	-104NS	-	334**	-
D. dilatata	61,13,13,62,61	1828***	360**	-222**	20NS	-	-254*	-
log(Al/Ca)								
P. elatior	40,13,13,40	-3.68***	-0.06NS	-0.22NS	-0.09NS	-	-	-
L. galeobdolon	29,9,9,29	-4.28***	0.06NS	0.06NS	-0.14NS	-	-	-
C. majalis	51,13,13,51	-1.99***	-0.10NS	0.47***	0.28*	-	-	-
D. dilatata	62,13,13,62	-1.12***	-0.16NS	0.30**	0.35**	-	-	-
log(N/P)								
P. elatior	40,13,13,40	2.14***	-0.11NS	-0.11*	0.10NS	-	-	-
L. galeobdolon	28,9,9,28,18	1.98***	-0.43**	-0.19*	0.48**	-	-	-0.39**
C. majalis	50,13,13,50	2.15***	0.06NS	-0.39***	-0.15*	-	-	-
D. dilatata	62,13,13,62	2.03***	-0.17**	-0.10**	0.004NS	-	-	-

-: variable excluded by model selection; NS: not significant; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Table 6.5 Parameter estimates of LMM’s explaining plant total content of Al, Ca, Mg, K, Kj-N and P with baseline set as TS (tree species)= *Alnus*, Light= shaded, and Forest= Eeuwenhout. AFS are ordered by increasing acid tolerance and shade tolerance (see Table 6.1).

	df	Intercept	TS	Light	Forest	TS x Forest
Al (mg)						
C. majalis	48,13,13,48	6.26NS	1.00NS	13.89***	5.72NS	-
D. dilatata	62,13,13,62	40.19***	0.46NS	43.75***	14.90NS	-
Ca (mg)						
C. majalis	48,12,12,48,12	59.85***	-11.41NS	32.72***	-15.27NS	34.05*
D. dilatata	62,13,13,62	113.31***	19.44NS	53.34***	-13.63NS	-
Mg (mg)						
C. majalis	48,13,13,48	11.81***	-0.26NS	8.54***	4.53*	-
D. dilatata	62,13,13,62	41.8***	9.5NS	21.2***	1.7NS	-
K (mg)						
C. majalis	48,12,12,48,12	124.66***	-31.94*	33.34**	-14.90NS	50.82*
D. dilatata	62,12,12,62,12	205.66***	-17.56NS	40.75**	-34.38NS	60.46*
Kj-N (mg)						
C. majalis	47,13,13,47	162.95***	5.01NS	17.12NS	-9.85NS	-
D. dilatata	62,13,13,62	310.37***	-24.56NS	31.19NS	12.99NS	-
P (mg)						
C. majalis	48,13,13,48	19.54***	-0.82NS	11.84***	2.06NS	-
D. dilatata	61,13,13,62	41.04***	4.59NS	9.25**	0.83NS	-

-: variable excluded by model selection; NS: not significant; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

6.4 Discussion

Clear tree species effects were found on the soil variables, survival and growth of the different AFS. Furthermore, some tree species effects are found on the plant nutrient concentrations and allocation. First, I will briefly discuss the trees species effect on the soil. Secondly, an answer is formulated on the different hypotheses given in the introduction. Thereafter, I discuss the differences between plant nutrient concentration and total plant nutrient content and I end with more general conclusions.

Table 6.6 Parameter estimates of LMM's explaining the ion shoot/root ratio between the above and below ground plant concentrations of Ca, Mg and Al with baseline set as TS (tree species)= *Alnus*, Light= shaded and Forest= Eeuwenhout. AFS are ordered by increasing acid tolerance and shade tolerance (see Table 6.1).

	df	Intercept	TS	Light	Forest	TS x Forest
log(Ca shoot/root ratio)						
C. majalis	51,13,13,51	1.05^{***}	-0.05NS	0.14^{**}	-0.02NS	-
D. dilatata	62,13,13,62	0.37^{***}	-0.07NS	0.25^{***}	0.02NS	-
log(Mg shoot/root ratio)						
C. majalis	51,13,13,51	0.50^{***}	-0.06NS	-0.18^{***}	0.09^{*}	-
D. dilatata	62,12,12,62,12	0.48^{***}	-0.02NS	-0.04NS	0.26^{**}	-0.23^{*}
log(Al shoot/root ratio)						
C. majalis	51,12,12,51,12	-3.53^{***}	0.13NS	-0.46^{***}	-0.03NS	-0.49^{*}
D. dilatata	62,12,12,62,12	-3.29^{***}	-0.34NS	-0.32^{**}	0.86^{***}	-0.97^{**}

-: variable excluded by model selection; NS: not significant; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Similar to previous studies (Cote and Fyles 1994; De Schrijver et al. 2012a), I found that the soil pH-KCl and pH-H_20 of the *Alnus* plots were nearly a full unit lower than the Fraxinus plots, planted on similar sites two or four decades ago. Despite the fact that the soil of Mortagne forest has a bit more silt and should be better buffered against acidification, the pH-KCl and pH-H_20 of this forest is about a full unit lower for both tree species. Eeuwenhout was planted almost two decades after the Mortagne forest, which at least partly explains the contrast. The trees have thus not yet acidified the former arable land of Eeuwenhout up to the level observed in Mortagne forest (De Schrijver et al. 2012a). Soil Ca and Mg concentrations correlate positively with pH-KCl and pH-H_20 while the opposite is found for the Al concentration. Especially the Al concentrations in the *Alnus* plots in Mortagne forest are very high compared to the other plots, explained by the soil acidity reaching the Al exchange buffer range. De Schrijver et al. (2012a) found N-accumulation resulting from this process only to be present in the older afforestations. Also in our plots, there is a strongly increased Kj-N concentration under the *Alnus* trees in Mortagne forest, whereas this was not yet the case in Eeuwenhout. The same pattern is found for the C concentration, whereas the soil C/N ratio merely depends on the tree species and not on forest age. There are some differences in the total P concentration of the different plots that are most likely explained by differences in historic fertilisation (De Schrijver et al. 2012b; MacDonald et al. 2012). The tree species effect on the bio-availability of P is explained by the altered pH-H_20 (Stevenson and Cole 1999).

The four selected species that are intolerant or intermediately tolerant to soil acidity, in general performed less in the acidified soil of *Alnus* for survival, vegetative and generative performance as well as for the weight after/before ratio. By contrast, the growth and survival of *C. majalis* and *D. dilatata*, i.e. the two acid tolerant species, were barely affected by tree species or forest. The total Al concentrations in *D. dilatata* and in some cases *C. majalis* exceeded the threshold of 1000 mg Al.kg^{-1} suggesting that these plants are Al accumulators (i.e. Al tolerant plant species that inhibit Al toxicity by complexation, Metali et al. 2012), while concentrations in *P. elatior* and *L. galeobdolon* were clearly below this threshold. The high Al concentrations in the roots indicate that both acid tolerant herb species store toxic Al primarily in the roots. The high soil Al concentration under *Alnus* in Mortagne forest has not resulted in a higher total plant Al concentration, but plants that suffered too much from Al toxicity died or reduced strongly in biomass. Individuals that died or had very low biomass could not be analysed, which may explain why no differences were found for the Al concentrations. Nevertheless, high soil Al concentrations did alter the ion shoot/root ratio by increased concentrations in the leaves. Ca concentrations of all species, except *P. elatior*, seemed to be lower in plants from the soil of *Alnus*. Also the allocation of Mg was effected by the acid soil of *Alnus* for *D. dilatata*. The differences in Ca and Mg concentrations and allocations can indicate either limited base cation supply (e.g. Falkengren-Grerup et al. 1995a) or an Al induced inhibition of the Ca uptake (e.g. Huang et al. 1992; Falkengren-Grerup and Tyler 1993) and base cation allocation (Ca allocation, Jones and Kochian 1995). Falkengren-Grerup Falkengren-Grerup and Tyler (1993) demonstrated that increasing pH-H_2O with SrCO3 had similar effects as CaCO3 and MgCO3 on the recruitment of 13 different forest plants via seeds. It was concluded that the H^+ activity, possibly aggravated by Al, rather than low cation levels, were responsible for the differences in recruitment success. For tree saplings, acid intolerance is also associated with Al rather than with low base concentrations. An elevated Al availability causes Al antagonism, root damage, Al accumulation within the root tissues and P deficiency (Weber-Blaschke et al. 2002). Abedi et al. (2012) further revealed that Al is one of the strongest restrictions to grassland species' occurrences in acidic soils.

Plant N concentrations are not lower in soil of *Fraxinus* revealing that there is no N limitation compared to the soil of N_2-fixating *Alnus*, despite the fact that N/P ratios are often below 10 which could indicate N limitation (Gusewell 2004). Higher plant P concentration and consequently lower N/P ratio in soil of *Fraxinus* does not match with the bio-P in the different soils. I estimate that bio-P is not limiting (plant P concentration of *P. elatior* similar to Baeten et al. (2010)), but differences are more likely explained by an antagonism between P and Al (Weber-Blaschke et al. 2002).

While light mainly affected vegetative performance and plant weight ratio, the soil influenced survival and generative performance. Despite significant effects of the light treatment on the plants, this is not in accordance with their spring or summer phenology as found by Baeten et al. (2010) and Verstraeten (2013). A reduction of the light level to 22% (mean in summer of the first year) might have been too low to trigger a strong response from these shade tolerant forest species. The light level was further reduced to 9% in the second year which might have been too late to trigger a reaction in the same year. Within these two years, the experiment did not reveal clear evidence for compensation in survival or growth for unfavourable soil conditions by increased light availability. However, soil acidification affected nutrient uptake, while the light treatment had a clear effect on the allocation of nutrients between below and above ground parts which might indicate an interaction on the long term. AFS have in general a shade tolerant or shade avoiding strategy (Hermy et al. 1999). Therefore, these species adopt their growth only moderately when light levels increase. This possibly explains the absence of a strong interaction as was found for light demanding grasses and tree species (Moir 1966; Portsmuth and Niinemets 2007). Future research on light compensation effects should pay attention to other forms of adaptation than growth, for example time of spring emergence.

The nutrient concentrations in the plants are in general lower in the unshaded treatment. However the total nutrient content increased in the unshaded treatment (Table 6.5). This means that dry weight increase was only partly followed by nutrient uptake resulting in growth induced dilution of the nutrients (Jarrell and Beverly 1981; Hipps et al. 2005) which might be further explained by a change in leaf architecture (Poorter et al. 2009). Poorter et al. (2009) clarified that light exposed plants have in general thicker leaves changing the correlation between biomass and leaf area.

Differences between herb species might partly be explained by their varying origin, nursery vs. collected in a forest. Nursery plants have been grown in potting soil, receiving optimal nutrient and soil acidity levels. I assume that these species had raised nutrient concentrations at the beginning of the experiment which could compensate for the unsuitable soil conditions during the experiment. For example, the nursery grown *P. elatior* could withstand the acidification much better than *L. galeobdolon* and *M. perennis*. It leaves little doubt that this was partly explained by the large plants of *P. elatior* and their nursery origin. Therefore, it would have been better that all plants had received similar pre-treatment but the choice in nursery plants was limited.

The soil organic matter content will increase in aging post-agricultural forests. This may benefit ancient forest plants, e.g. via the increased water holding capacity of the soil. However, soil pH and nutrient availability might decrease over time which disfavours the performance of many species. I found that survival and growth was in general lower in the older Mortagne forest than in the younger Eeuwenhout, thus increased organic matter does not compensate for the increased acidity in older post-agricultural forest.

I conclude that tree species have a clear effect on the soil acidification which, in turn, influences the survival and growth of herb layer species. This was most likely explained by Al toxicity, resulting in lower plant Ca and P concentrations (Al antagonism) and differences in allocation of Al and Mg between root stocks and leaves. Furthermore, the herb layer is affected by a differential shade casting of the tree species. I found no light compensation for the soil effect on the species survival and growth, however, light altered the nutrient concentrations and allocation. This could indicate an indirect effect on the long term. Therefore, it would be interesting to study the survival and growth and plant element concentrations in long-term field experiments.

7

General discussion and conclusions

The distribution of so called AFS has been explained to be limited by both dispersal and environmental limitation. Environmental limitation in post-agricultural forests is in general related to elevated soil P concentrations and altered competition between AFS and P-opportunistic competitive species. These competitors become dominant as a result of the elevated soil P-levels, a legacy of the former land use, in combination with a sufficiently high light availability.

This thesis focuses on options for forest management to mitigate or overcome the environmental limitation of these species. Therefore, the effect of the tree species choice on the herb layer development is studied, since the tree species show a great impact upon the forest ecosystem.

In general, the results lead to the conclusion that tree species are important drivers of the understory development in post-agricultural forests (objective 1, see chapter 1), in particular through their impacts on soil acidification and understory light availability (objective 4). In immature post-agricultural stands, tree species show to have a diverging effect on soil acidity, within a period of only 20 to 30 yr (objective 2, for more discussion see chapter 2).

In the following discussion and conclusions, I will first focus on the differential mechanisms explaining tree species effects (objective 4). Then, I discuss the specific differences across different life stages of the AFS (objective 3). Thereafter, I will present a conceptual framework on tree species effects, which makes it possible for forest managers to use tree species effects to steer the herb layer development (objective 5). Finally, I give some perspectives for further research.

7.1 Tree species effects

7.1.1 Tree species effect explained by soil effects

The differential soil acidification between tree species is found to be one of the major factors explaining tree species effects on herb layer development. Broadleaved tree species can be grouped in two rather separated groups, soil-acidifying species that quickly cause the soil to reach the Al buffer range and species that only moderately acidify the soil (Neirynck et al. 2000; Reich et al. 2005; van Oijen et al. 2005; chapter 3). Starting from agricultural land, the soil generally has a near neutral pH, but soil acidifying tree species have a strong and rapid effect lowering the pH in only about 20 to 30 yr to the Al buffer range (De Schrijver et al. 2012a; chapter 2). Likely, the decrease in pH does not happen gradually but quite suddenly within a period of only a few years when the base saturation is becoming low. The pH then decreases rapidly till a new equilibrium is reached in the Al buffer range. At the same time, litter starts to accumulate. This is explained by the self-retarding impact of poor litter quality. A low litter Ca concentration negatively affects earthworm populations and other litter fauna. A decline in the population and diversity of these litter decomposers, retards the litter decomposition and results in litter accumulation. Through this process the main decomposers shifts from invertebrates towards fungi. Increasing decomposition by fungi increases the production of organic acids which again limits the litter fauna (Hommel et al. 2002; Reich et al. 2005; De Schrijver et al. 2012a).

The second group of tree species seems to show some range from species that are able to maintain the high soil pH of the arable land (e.g. *Populus* spp.) up to species that moderately acidify the soil just above the Al buffer range (e.g. *A. pseudoplatanus*). van Oijen et al. (2005) even considers *A. pseudoplatanus* to be in a third group of intermediate species, but in the study of Reich et al. (2005) *A. pseudoplatanus* and *Acer platanoides* L. have nearly the highest soil pH and litter Ca concentration. The litter quality of a particular species also depends on the soil conditions (e.g. van Oijen et al. 2005; Kooijman 2010) and other factors which might explain the differences found between studies. Therefore, giving a precise sequence of tree species litter quality is difficult (Barbier et al. 2008).

In all the studies presented in this thesis, soil acidification by tree species influenced the abundance, survival, growth and/or germination of some of the AFS. In most cases acid intolerant AFS did not survive in soil pH within the Al buffer range. In chapter 6, the increased soil Al concentration was found to affect plant Ca concentrations and Mg and Al shoot/root ratios. Therefore, Al toxicity likely explains the low survival of acid-intolerant AFS under soil acidifying tree species. Acid tolerant species seem to germinate better in moderately acidified soils (chapter 4), but their survival and growth was independent with respect to acidification in (chapter 3 and 6). In natural circumstances (chapter 2), acid tolerant species were more abundant on acid soil. This shows the tolerant nature of these species, rather than a preference for acid soils. Likely, they grow as good as or even better on moderately acidified soils but are outcompeted here in natural circumstances. Therefore, it is concluded that planting acidifying tree species in post-agricultural forest excludes acid sensitive AFS within a few decades after afforestation.

Litter accumulation is associated with soil acidification and, therefore, the exact nature of litter effects should be studied by litter removal or addition experiments. Within this thesis litter was always studied in combination with other soil acidifying effects. Litter removal experiments were not carried out. Therefore, I refer to the discussion of chapter 3 for a discussion on published litter removal experiments.

The share of soil P that is bio-available is affected by the pH (lower in acid soils) and thus by tree species (chapter 2 and 5). However, lower pH with lower levels of bio P do not suppress competitors (for example chapter 1 and 2, see also further). This is likely explained by the fluxes between P pools (Stevenson and Cole 1999) and a large yearly input of P to the top soil by tree litter decomposition (3.1 kg P $.ha^{-1}.yr^{-1}$ according to De Schrijver et al. 2012b). Therefore, limiting P availability by soil acidification is not a plausible strategy to limit competitors.

These results were studied on acidification sensitive soils where tree species have a predominant influence on the soil pH. On sandy soils with a very poor buffering capacity against acidification, a limed post-agricultural soil will become acid regardless of the tree species used, especially under the current acid deposition. The typical herb layer species of sandy soils like *M. bifolium, C. majalis, Teucrium scorodonia* L. and *P. aquilinum* are acid tolerant. Soils that are rich in free Ca, e.g. in sites where base rich source water constantly resets the acidification or on heavy clay soils with a high CEC and base saturation, again the tree species will have little effect on the soil acidity and the colonisation of acid intolerant species.

7.1.2 Tree species effect explained by light effects

Throughout this thesis, light transmittance was most often simplified to a single value based on the mean transmittance in the summer. Nevertheless, effects of differential light transmittance caused by differences in tree species were found (chapter 2 and 3). AFS establish better under trees with moderately light levels, while their growth is improved by lower light levels which suppresses competitors (chapter 3). Light demanding AFS were mainly found under the higher light transmittance of *Populus* (chapter 2).

Temporary gaps in the canopy cover are suitable for the recruitment of more competitive and taller AFS like grasses and ferns (De Keersmaeker et al. 2011; chapter 3). While performance of small summer green hemicryptophytes on the clearcut was lower than in the other stands (chapter 3). If the gaps close quickly enough, competitors cannot become dominant. When the canopy is closed again, the low levels of light are still suitable for the growth of AFS.

The results of chapter 5 are not yet conclusive but it might be expected that also the difference in leafing phenology between different shrub or tree species has an influence on the herb layer assemblage.

7.2 Colonisation success across different life stages or age classes

Chapter 4 and 5 report on seed sowing experiments that are evaluated for one or two years, while chapter 3 reports on an introduction of juveniles ready for flowering. Here, I will try to join these different chapters and give an overview across life stages and age classes. For this, I will focus on three AFS that are used in all of these chapters, and two scenarios, an acidifying tree species and a moderately acidifying tree species (excluding the clearcut). Germination and survival during the first year can be compiled either from the results of chapter 4 or 5. The results of chapter 4 are much higher which is logically explained by the fact that seeds were sown in a more protective environment. To allow calculations later, I changed 0% germination in ‘<<1%’. The seeded plants of *P. elatior* and *H. non-scripta* used for the introduction are estimated to be about 4 yr old. For *A. nemorosa*, the plants resulted from vegetative reproduction but based on their size are estimated to accord to plants of a similar age (cf. Ernst 1983; Shirreffs 1985).

Under the moderately acidifying tree species, the survival rates seem to increase with the age of the introduced population (cf. Ehrlén and Eriksson 2000). Under these tree species, the survival rates of the three AFS are all above 95% for the adult plants of 9 to 13 yr of introduction (Table 7.1). While under acidifying tree species, the number of populations still strongly decline, e.g. half of the *P. elatior* populations lost yearly under an acidifying tree species after 9 to 13 yr of introduction. This strong decline likely means that with natural colonisation these plants would never have developed so far from seeds which fits the absent germination.

7.3 Long term prospects for ancient forest species colonisation

If the survival rates are summoned together for the entire period of 13 yr, it results in 2 to 3% survival under the moderately acidifying tree species (i.e. 2 to 3 populations resulting from the introduction of 100 seeds). In contrast, overall survival rates for *P. elatior* and *A. nemorosa* under the acidifying trees are smaller than 0.04% (4 populations for every 10000 seeds). Via spontaneous colonisation, these plants will, likely, never colonise under these tree species. In contrast, survival rates of *H. non-scripta* are not influenced by tree species. These results strongly depend on the initial germination which might be considered as a bottleneck for the recruitment of these species (Baeten 2010). The germination in pots in chapter 4 was remarkably higher, while the in situ experiment in chapter 4 resulted in an extremely low recruitment. Also between tree species groups, the largest difference is found for germination.

Table 7.1 Yearly survival rates (%) of AFS across different age classes and two tree species scenarios (TS, MOAC: Moderately acidifying tree species; ACID: Acidifying tree species) in post-agricultural forest. Values between brackets are estimated values based on means between adjacent age classes.

		Ch 4	Ch 5			Ch 3			
AFS	TS	1 yr	1 yr	2 yr	3/4 yr	5/6 yr	7/8 yr	9/13 yr	1-13 yr
P. elatior	MOAC	65	12	67	(80)	93[a]	94	96	3[b]
	ACID	<<1	-	(86)	(86)	86[a]	45	58	<<0.006
A. nemorosa	MOAC	5	6	(89)	(89)	89	86	98	2
	ACID	<<1	-	(79)	(79)	79	53	85	<<0.04
H. non-scripta	MOAC	10	16	75	(84)	92	92	95	3
	ACID	10	-	(89)	(89)	89	89	88	2

[a]: For *P. elatior* the results of chapter 3 are based on the introduction of a cluster of plants;
[b]: $=0.12*0.67*(0.80)^2*(0.93)^2*(0.94)^2*(0.96)^5$.

After initial establishment, the survival is fairly high which confirms the survival strategy of AFS rather than a colonisation strategy (Brown and Oosterhuis 1981; Grashof-bokdam 1997). Consequently, their future survival might remain rather stable. For example, Van Der Veken et al. (2007) found a survival of 41% of adult *H. non-scripta* transplants after 45 yr introduction in ancient forest. This is comparable to the results of chapter 3 after 9 yr introduction. This might be explained as a difference between survival in ancient versus post-agricultural forests or as the first decade is crucial for initial establishment and survival. However, many of the populations are still small (because of limited vegetative or generative spread) making them very vulnerable for local disturbances. Furthermore, the problem of the high soil P amount is not solved and populations might quickly disappear after intensive tinning or clearcuts due to strong reaction of competitors.

Consequently, future colonisation might depend mainly on the expansion of the individual populations. The introduced individuals grew out to a mean cover of 118 (minimal 13 to maximal 755) cm² for *A. nemorosa* which has the lowest cover up to 0.7 m² (70 cm² - 3.3 m²) for *M. perennis*. Van Der Veken (2007) found maximal 26 m² after 45 yr for *H. non-scripta*. Based on this, it is clear that the colonization of AFS takes many decades up to a century even with the best allocation or introduction and management (cf. Bossuyt and Hermy 2000; Honnay et al. 2002a). The selection of the tree species must, therefore, be seen in the light of creating ideal circumstances to allow a slow but steady colonisation while an unsuitable environment might lead to absent or extremely slow colonisation.

This slow restoration, furthermore, underlines the irreplaceable value of ancient forests. Consequently, safeguarding AFS should firstly focus on protecting ancient forest.

7.4 Conceptual framework

I conclude that tree species effects on herb layer development are mainly explained by two factors: light transmittance and soil acidification. Consequently, each tree (or site) can be represented in a two dimensional plot to generate a conceptual framework which allows to understand the vegetation development and generate management guidelines. As the soil acidification had the strongest impact in our experiments, it is represented on the x-axis and light transmittance on the y-axis. Figure 7.1 positions each research plot of chapter 2, 3 and 5 along these two axes, also showing the tree species in the respective plots. Note that the variation that is presented for a given tree species also depends on the number of plots, the number of stands and the variable density of the shrub layer. Further, *A. glutinosa* from Mortagne forest is positioned at an odd place. In chapter 3, *A. glutinosa* was grouped within the light rich stands because of the high amount of lateral light that falls in this stand at the southern border of the forest. This light is captured with the LAI meter but not by the densitometer which has a rather narrow angle. Here, the results of the densiometer are used as these measurements are available from all chapters.

To outline the zone which is favourable for the colonisation of AFS, thresholds must be drawn for pH-H_20 and light transmittance. For pH-H_2O, this can be set on 4.2 where the Al-buffer range is reached (Stevenson and Cole 1999; Fisher and Binkley 2000; Lukac and Godbold 2011). For light transmittance, the threshold has not been set in previous chapters. De Keersmaeker et al. (2004) set a threshold at 8% light transmittance for suppressing competitors and allowing AFS to colonise. A light level of about 10% also demarks the strongly shaded stands from chapter 3 which was associated with increased cover of introduced AFS. For the data of chapter 2 and 3, a level of 8% light strongly limits the cover of the main competitors, i.e.: *U. dioica, Rubus fruticosus* L. and non-forest grasses (grasses, excluding typical forest plant species based on Honnay et al. 1998 annex 1) (Figure 7.2). In 43% of the represented plots of chapter 2 and 3, one of these competitive groups exceeds 30% (treshold based on the criteria for a well conserved forest habitat status, T'Jollyn et al. 2009). In the plots with less than 8% light transmittance, this is only the case in 21% of the plots, while in plots having between 8 and 20% light transmittance, competitors exceed 30% cover in 79% of the cases. Furthermore, if the *A. glutinosa* stand is removed (see discussion in previous paragraph), only 19% of the dark stands have an abundant cover of competitors. The cover of all of the introduced AFS (data of chapter 3) is higher in sites with light transmittance below 8% compared to sites with higher light transmittance but differences are not significant (Figure 7.3). In contrast, the survival (establishment) of three of these species, i.e. *A. nemorosa*, *M. perennis* and *O. acetosella*, are significantly higher (respectively z = 3.226, p = **, z = 3.225, p = ** and z = 4.367, p = *** with df = 1,175) under moderately levels of shade (8-20%) than under strong shade (≤8%): respectively 42 compared to 18%, 22 compared to 5% and 32 compared to 2%. Data of chapter 2 does not allow comparison as not enough stands adjacent to ancient forest are included in both light levels. To conclude, 8% light transmittance can be used as threshold to suppress competitors and facilitate growth of competitors but higher light transmittance might improve the establishment of some AFS.

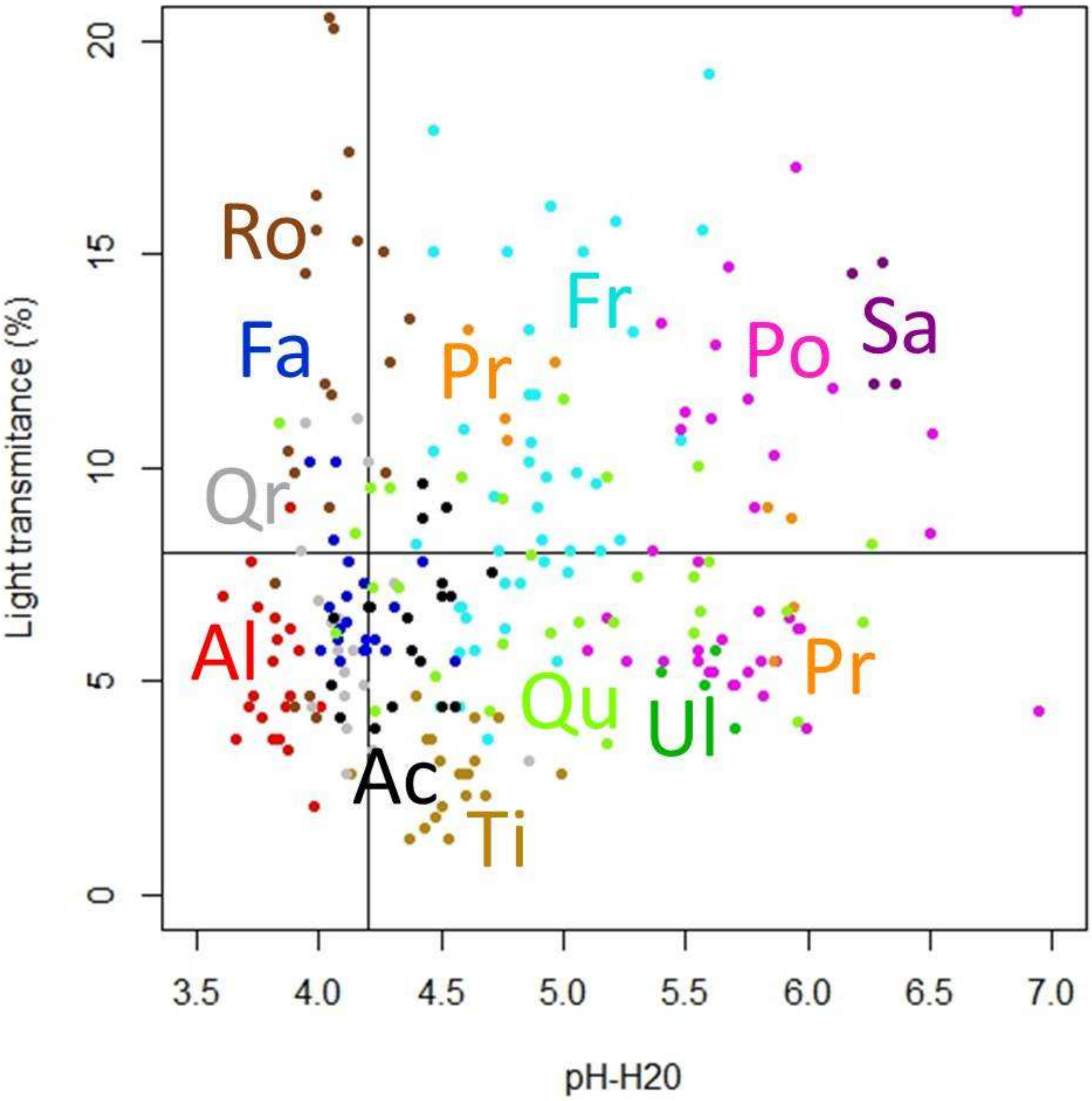


Figure 7.1 Position of tree species represented by pH-H_2O of the topsoil and light transmittance (measured with densitometer) of chapter 2, 3 and 5. Stands with more than 20% light transmittance (young stands, clearcut) are not represented. Ac: *Acer pseudoplatanus*, Al: *Alnus glutinosa*, Fa: *Fagus sylvatica*, Fr: *Fraxinus americana*, Po: *Populus* x *canadensis* and *P.* x *generosa*, Pr: *Prunus avium*, Qu: *Quercus robur* and *Q. petraea*, Qr: *Quercus rubra*, Ro: *Robinia pseudoacacia*, Sa: *Salix alba-fragilis- x rubens*, Ti: *Tilia cordata* and Ul: *Ulmus x hollandica*. Black lines represent thresholds of 8% light transmittance and pH-H_2O 4.2.

This framework can now be used to plot, for example, tree species, site data or management scenarios to give an easy interpretation of the herb layer restoration potential (Figure 7.4). When seed sources of forest species are absent, sites in quadrant I and IV will often have no or only a sparingly vegetation cover. If seed sources are available, forest species will slowly colonise but in quadrant IV the species pool will be limited to acid tolerant species. In quadrant II the vegetation is most often dominated by *U. dioica* or grasses. The herb layer in quadrant III is either dominated by *R. fruticosus, U. dioica* or grasses. The sides dominated by *R. fruticosus* are the more acid or more shade rich (just above 8%) sites. Besides pH-H_2O and light availability, probably also some historical land use and management factors might have an effect on which competitor dominates a plot.

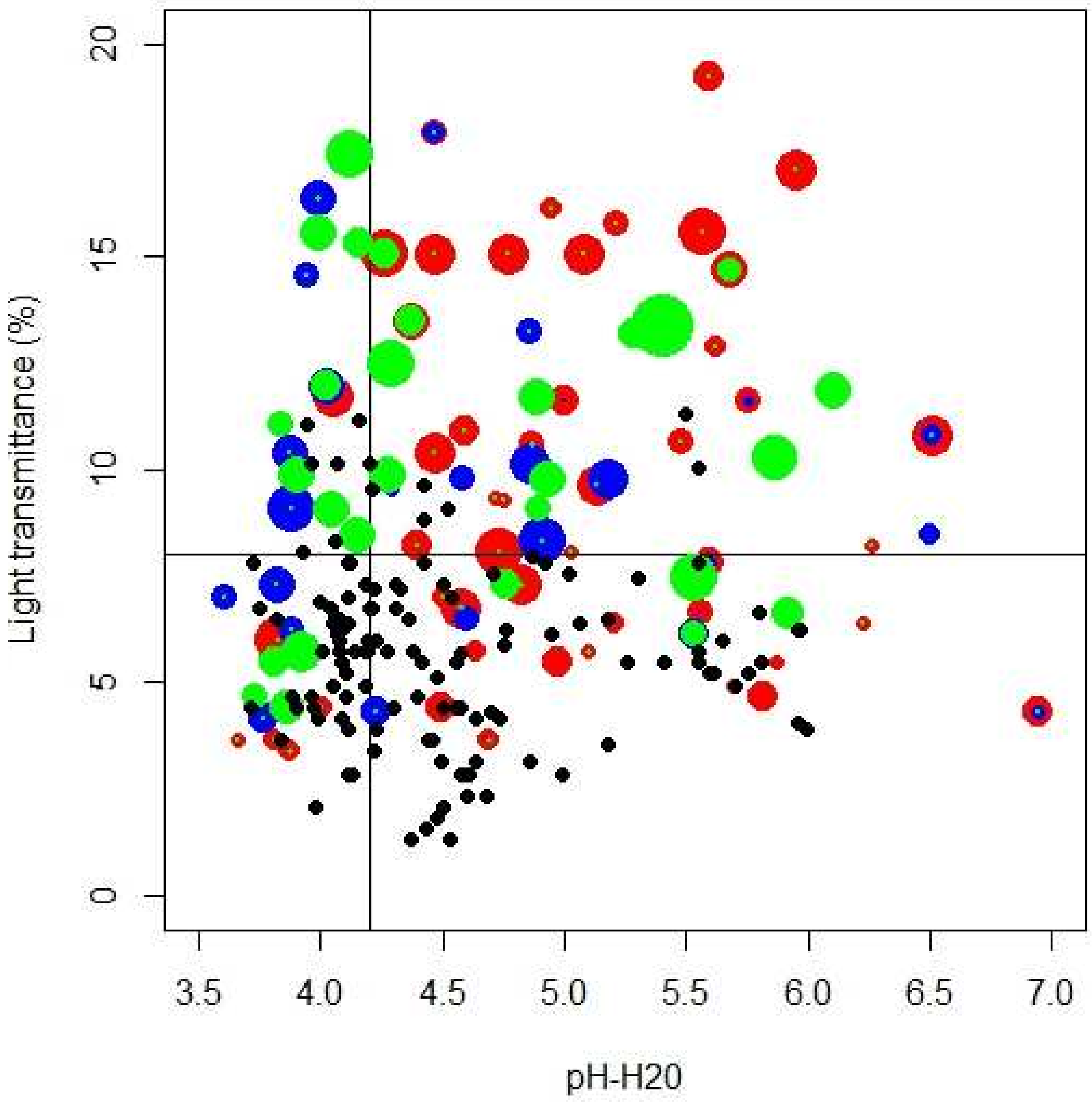


Figure 7.2 Cover of *Urtica dioica* (size of red circles, range between 0-90%), *Rubus fruticosus* (size of blue circles, range between 0-90%) and non-forest grasses (size of green circles, range between 0-120%) positioned by topsoil pH-H_2O and light transmittance of plots of chapter 2 and 3. Black circles represent plots were none of the competitors reach 30% or more. Black lines represent thresholds of 8% light transmittance and pH-H_2O 4.2.

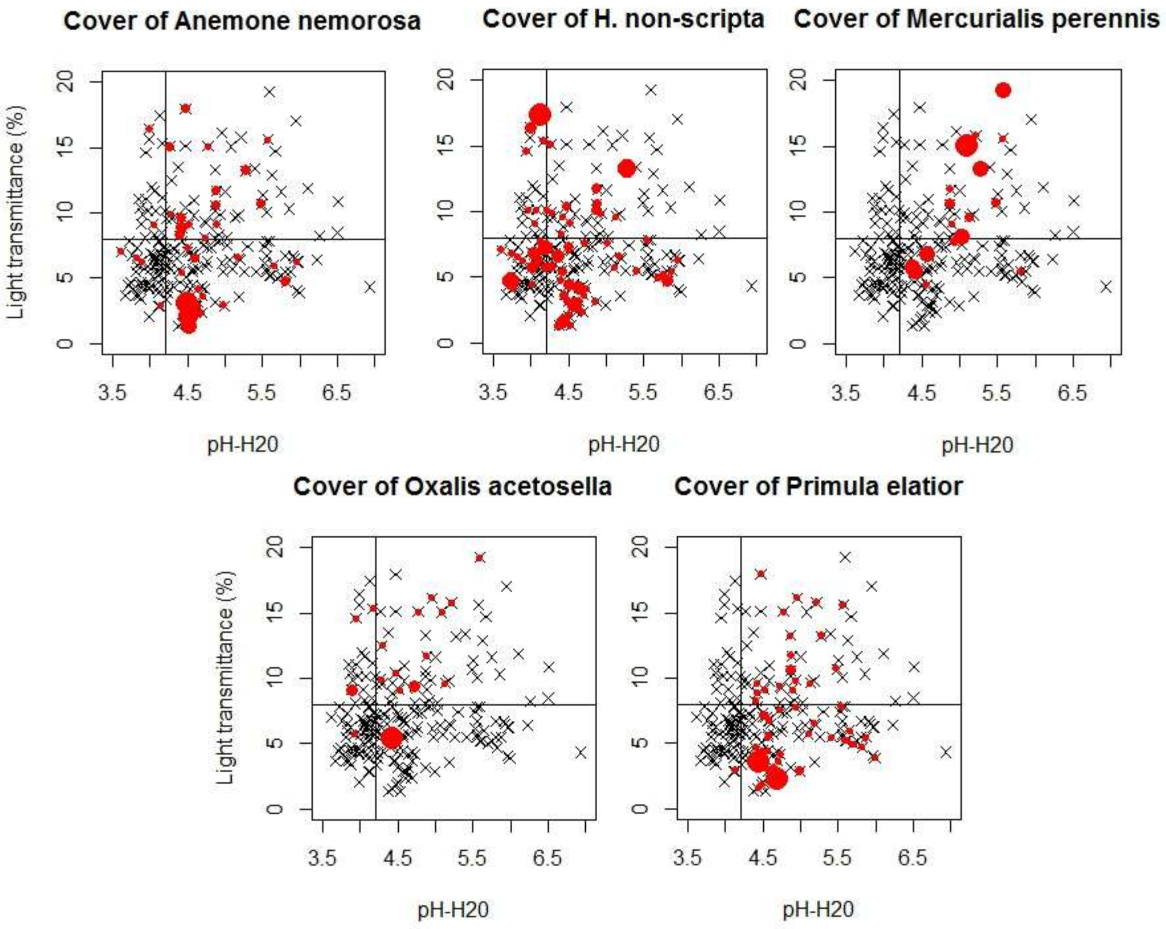


Figure 7.3 Cover of introduced plants of *Anemone nemorosa*, *Hyacinthoides non-scripta*, *Mercurialis perennis*, *Oxalis acetosella* and *Primula elatior* of chapter 3 positioned by topsoil pH-H_2O and light transmittance. Black lines represent thresholds of 8% light transmittance and pH-H_2O 4.2.

7.5 Conclusions for forest management

7.5.1 Tree species selection

Tree species effects can be evaluated by two thresholds, distinguishing four different groups of tree species represented by the different quadrants (Figure 7.4). Some tree species are positioned in this figure based on the data of chapter 2, 3 and 5. Some other important trees species could be added: *C. betulus* generates a moderately acidified soil and should be placed in quadrant I as it creates deep shade (e.g. Le Duc and Havill 1998). Litter quality of *C. sativa* was just a bit better than *Q. robur* in a Swiss study (Sariyildiz and Anderson 2003), but in general litter of this species strongly accumulates in our regions. The summer light transmittance of *C. sativa* can be less than 1% under closed coppice (Mason and MacDonald 2002). Therefore, *C. sativa* is positioned in quadrant IV. With few exceptions soil acidification under conifers is even stronger than under acidifying broadleaves (Barbier et al. 2008) and light transmittance can be low or high, but mainly differ with broadleaves in phenology as most of them are evergreen (e.g. Verstraeten et al. 2012).

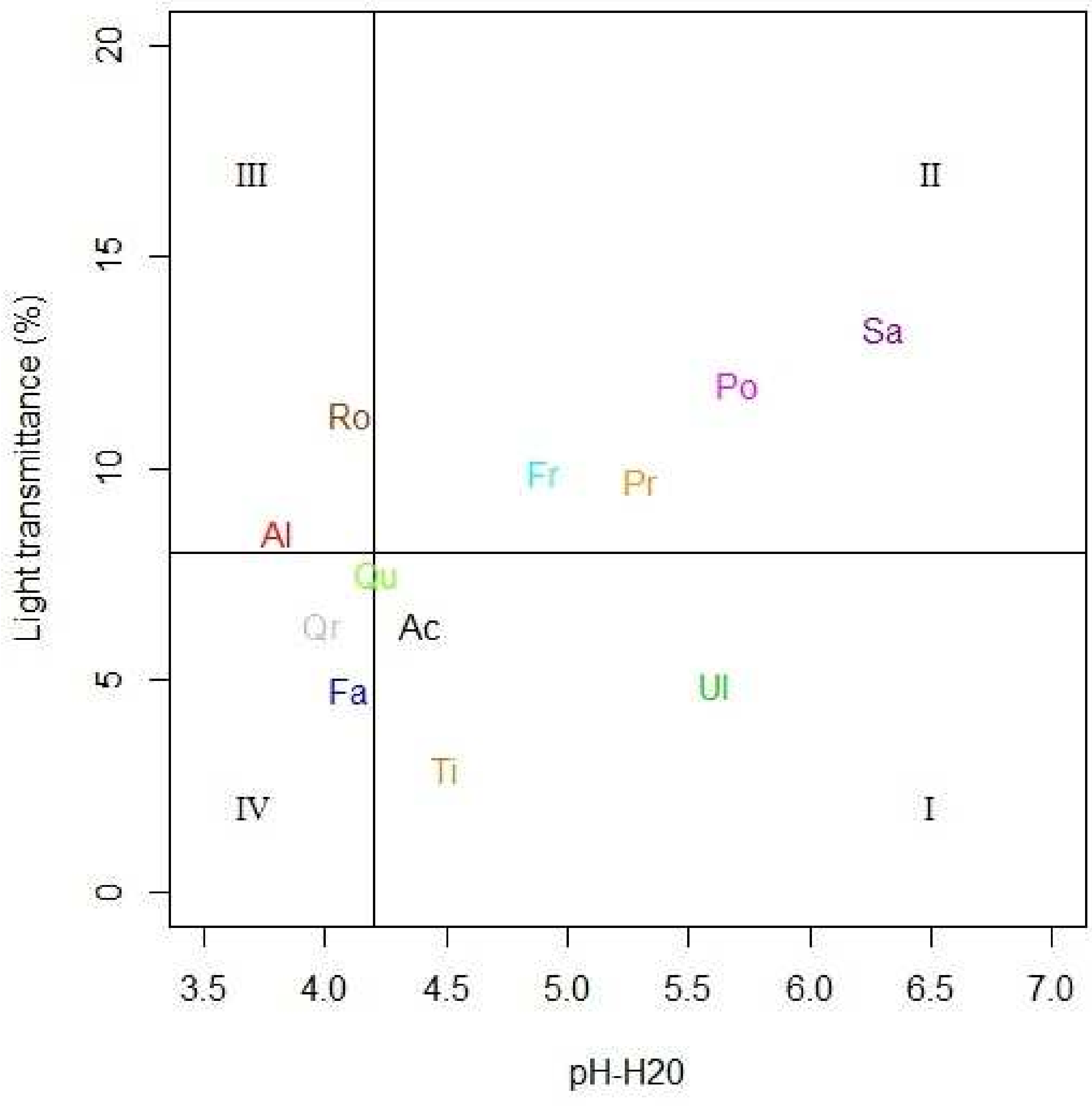


Figure 7.4 General framework to assess tree species effects by topsoil pH-H_2O and light transmittance. Abbreviations see Figure 7.1. Black lines represent thresholds of 8% light transmittance and pH-H_2O 4.2.

To stimulate the colonisation of different AFS, tree species in quadrant I, i.e.: *Tilia spp.*, *Ulmus spp.*, *C. betulus* and *A. pseudoplatanus* can be used. Furthermore, tree species of quadrant II, i.e.: *Populus spp., Salix spp., F. excelsior* and *P. avium* are usable when more shade is generated by means of a shrub layer. For example, the *Populus* stand of Mortagne forest is positioned in quadrant I due to the shrub layer of *C. sativa*. Using these moderately shading tree species gives more possibility's to manage the light regime and to create temporary gaps. Shade of a tree species of quadrant II can be controlled by the species and densities of shrubs that are planted and by (partly) coppicing the shrub layer. In this way, gradients in shade can be created in space and time within the stand. These gradients generate different areas or periods that are more suitable for either recruitment or growth of AFS.

Tree species of quadrant III and IV generate acid soils which limit the pool of AFS that can colonise and, therefore, it should be avoided to plant these species in homogenous stands.

7.5.2 Tree species mixtures and stem density

The research focused on single tree species effects while tree species mixture is more and more promoted in close-to-nature forestry. Furthermore, *Quercus* spp. and *F. sylvatica* are frequently used and economically important tree species, but both fall within quadrant IV for which it was advised to not plant them in homogeneous stands. Therefore, I will explore here the possibilities of tree species mixtures and increasing stem density to reduce soil acidification.

The ratio of the litter amount (or basal area as a proxy) between trees species is found to be determining the canopy effect on soil acidity and herb layer diversity (e.g. Hicks 1980; Rothe and Binkley 2001; Langenbruch et al. 2012; Bartels and Chen 2013). A relation between tree diversity and understory diversity is often found, often explained as caused by increasing understory environmental gradients (e.g. Gilliam 2007; Barbier et al. 2008; Vockenhuber et al. 2011). However, many studies compare homogeneous stands of a soil acidifying tree species with mixed stands with a higher proportion of moderately acidifying tree species or conifers to mixed conifer-broadleaved forests. For example, Mölder et al. (2008) found an increasing herb layer diversity with increasing tree species richness but lower beech proportion had a more profound effect than the number of secondary tree species (but see Vockenhuber et al. 2011). Furthermore, the improvement of two species mixed stands over the homogeneous stands of each of the tree species has rarely been proven (Barbier et al. 2008). Consequently, it is unclear whether the proportion of moderately acidifying tree species rather than the tree species diversity is responsible for the understory diversity. Currently, many new studies are set up to investigate tree diversity effects (Baeten et al. 2013). Nevertheless, tree species from quadrant I and II need to be intermixed to keep the pH-H2O clearly above 4.2.

Furthermore, the percentage of tree species that needs to be intermixed to keep the pH-H2O above 4.2 might further depend on the level of acid deposition from air pollution and soil type (cf. Rothe and Binkley 2001; Thelin et al. 2002).

Species can be intermixed in different ways, from individual to stand wise. In Eeuwenhout (chapter 4), it was clear that even when these tree species are intermixed in small blocks (9x9 m), gradients in litter thickness and soil acidity develop between blocks rather than an stand wise improvement of the litter quality is reached. As trees age, the block wise single tree effects will likely evolve to a more homogeneous intermediate effect of the different tree species. In the QD-method (Landesforsten Rheinland-Pfalz s.d.; Wilhelm and Rieger 2013), the target species (for example *Quercus* spp. or *F. sylvatica*) is planted in small groups with a distance between groups equal to the distance between final crop trees. This method leaves large parts of the stand free for natural regeneration or for the planting of other tree species that fulfil other goals such as keeping the pH-H_2O above 4.2. Another possibility is to prevent soil acidification, is under planting of a shrub layer with good litter quality, like *C. avellana* (e.g. Mohr and Topp 2005).

Alternatively, *Quercus* spp. might also be planted at large spacing similar to *Populus* cultivars. This would reduce the initial litter fall and improve litter perturbation and decomposition (cf. Hobbie et al. 2006). Furthermore, it might generate lower canopy cover, at least in the first years, making it possible to plant or recruit a shrub layer.

7.5.3 Selecting other goals

When AFS are not present in the vicinity and introduction is not an objective, there is no use to manage the forest to stimulate their colonisation. Giving too much shade will result in an absent or only sparsely present herb layer. In these cases, it is better to aim at patches of *R. fruticosus*, *U. dioica* and rough *Arrhenatherion* grassland with *Apiaceae* and other flowers. Also this flora is species rich and serves various faunal biodiversity as shelter or food source. Even in these strongly isolated forests some forest species will eventually colonise. In the isolated Mortagne forest the colonisation of at least 15, often fast colonising, forest species is observed over the years: *A. filix-femina, C. lutetiana, C. sylvatica, D. carthusiana, D. dilatata, D. filix-mas, G. tetrahit, G. urbanum, H. helix, L. galeobdolon, P. multiflorum, R. ficaria, S. holostea, S. sylvatica* and *Viola odorata* L. (Thomaes and Vandekerkhove 2005).

7.6 Perspectives for further research

As the focus of this thesis lies upon the application rather than on the detailed understanding of tree species effects on herb layer development in post-agricultural forest, many possibilities for further in depth research remain. The soil impact has been studied in most detail here and together with many other publications, it is a well-documented factor. Fewer studies have been performed on the mechanical impact of the litter layer on herb layer species, especially in post-agricultural forest. However it is doubtful whether this would lead to altered conclusions since thick litter layers are linked to unfavourable soil acidification.

The effect of light availability is difficult to measure because of daily and tree species specific seasonal variation and hence less research has focused on this factor. As herb layer species differ in their photoactive period, these seasonal effects might have species specific effects. Chapter 5 shows first results in this way but more thorough studies on the differences in light seasonality are needed to understand this effect of leafing phenology and to fully explain this factor of tree species effects. The effects of various leafing phenology treatments on herb layer development and the capacity of different herb species to anticipate on the leafing phenology is an element for further research. Furthermore, the possibility of creating temporary canopy gaps for the recruitment of forest species seems a promising perspective for forest restoration. However, some research should focus on the optimal size, light availability and closure time of such gaps to avoid establishment of competitors and recruit AFS depending on the soil P concentration.

In chapter 3 and 5, I have studied the interaction between the soil and light factors of tree species effects but it was concluded that there was no interactive effect on the survival and growth of AFS. For tree saplings and light demanding grasses, interactions have been found. Therefore, it is interesting to study in more detail if and why these interactions are not found for AFS.

The research within this thesis was performed in homogeneous stand. Consequently, the question remains which canopy effects might be expected in mixed forest stands depending on tree species and type of mixture. Furthermore, I wonder to which extend tree species effects, like litter accumulation, soil acidification and light availability depend on planting densities. Again, some research has already been conducted but little in post-agricultural forests.

The primary source of the limited colonisation is the elevated P level in the soil. The shading effect of tree species mainly interacts with the abundance of competitors caused by the elevated P but it would be interesting to also study how forest management can directly impact the soil P concentration. P mining and topsoil removal or replacement are possible techniques but it is often suggested that these techniques are difficult to apply for forest ecosystems as tree roots bring P from larger depths compared to grassland species in the nutrient cycle, enriching the topsoil again. It is however interesting to look how much P is cycled in this way and how long it would take different tree species to enrich the topsoil with P or how deep topsoil needs to be removed and replaced with P poor soil to prevent the recycling of deeper P by different tree species. This knowledge on soil replacement might be especially interesting for soil translocation of ancient forests (including the AFS and other organisms) when such a forest, despite a proper protection, needs to be cleared (Helliwell et al. 1996). The latter authors found that many herb layer species can survive such a translocation. However, on P rich sites deeper soil layers will have to be replaced as well. Also the form in which P is stored in the soil (De Schrijver et al. 2012b) should be studied in more detail: does forest management impacts these P pools and is a different distribution of available soil P among pools meaningful in suppressing competitors? Furthermore, what is the role of these competitors, which can take up large quantities of P, in keeping P within a bio-available pool.

Root competition, as a possible factor of tree species effects on herb layer species, has not been studied in the context of this thesis. However, root competition between the tree layer and herb layer has been found (Coomes and Grubb 2000; Lindh et al. 2003; Powell and Bork 2006) and as tree species differ in rooting depth, it can be expected that tree species can have differential effects on the herb layer by root competition. In the post-agricultural forest like studied here, soil nutrients and water seems, however, not to be limiting factors, thus root competition might be of marginal significance.

Finally, I would like to plea for widening the research on AFS besides exploring it in deeper detail. Much research has already focused on ancient forest plant species. However, the strongly fragmented and disturbed forests in regions like Flanders do not only hamper the safeguarding of the vascular plant biodiversity. Likely, many other forest specialists are limited by either dispersal or environmental limitation and thus face difficulties colonising post-agricultural forests (e.g. carabid beetles: Desender et al. 1999; slugs: Kappes 2006; weevils: Buse 2012; bryophytes and lichens: Vanderpoorten et al. 2001; Fritz et al. 2008). However, these species are often ill studied because they are less easy to study than plants. However, new technologies that make research on such species easier are making progress. This creates new opportunities to test the detailed knowledge of the ecology of ancient forest plant species on other organisms and test the general validity of the inferred processes. Doing so, it will be possible to evaluate whether current guidelines for management also apply for these species.

Appendix A: Abbreviations used in Figure 2.2

Abbreviation	Name	Abbreviation	Name
Agrosto	*Agrostis stolonifera* L.	Holclan	*Holcus lanatus* L.
Alopgen	*Alopecurus geniculatus* L.	Junceff	*Juncus acutiflorus* Ehrh. ex Hoffmann
Anemnem	*Anemone nemorosa* L.	Lamigal	*Lamium galeobdolon* (L.) L.
Angesyl	*Angelica sylvestris* L.	Loniper	*Lonicera periclymenum* L.
Anthsyl	*Anthriscus sylvestris* (L.) Hoffmann	Milleff	*Milium effusum* L.
Arrhela	*Arrhenatherum elatius* (L.) Beauv ex J. et C. Presl	Phalaru	*Phalaris arundinacea* L.
Cirsarv	*Cirsium arvense* (L.) Scop.	Phlepra	*Phleum pratense* L.
Cirsole	*Cirsium oleraceum* (L.) Scop.	Poanem	*Poa nemoralis* L.
Clemvit	*Clematis vitalba* L.	Poapra	*Poa pratensis* L.
Dauccar	*Daucus carota* L.	Poatri	*Poa trivialis* L.
Dryodil	*Dryopteris dilatata* (Hoffmann) A. Gray	Ranufic	*Ranunculus ficaria* L.
Dryofil	*Dryopteris filix-mas* (L.) Schott	Ranurep	*Ranunculus repens* L.
Epilang	*Epilobium angustifolium* L.	Rubucae	*Rubus caesius* L.
Epilsp	*Epilobium* spp.	Rubufru	*Rubus fruticosus* group
Eupacan	*Eupatorium cannabinum* L.	Rumeobt	*Rumex* obtusifolius L.
Galetet	*Galeopsis tetrahit* L.	Stacsyl	*Stachys sylvatica* L.
Galiapa	*Galium aparine* L.	Stelhol	*Stellaria holostea* L.
Gerarob	*Geranium robertianum* L.	Taraoff	*Taraxacum officinale* group
Geumurb	*Geum urbanum* L.	Urtidio	*Urtica dioica* L.
Gleched	*Glechoma hederacea* L.	Vicisat	*Vicia sativa* L.
Herasph	*Heracleum sphondylium* L.	Waldter	*Waldsteinia ternata* (Stephan) Fritsch

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