Gorik Verstraeten

Conversion of deciduous forests to spruce plantations and back: evaluation of interacting effects on soil, forest floor, earthworm and understorey communities

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

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Dutch translation of the title:

Omvorming van gemengd loofbos naar fijnsparaanplantingen en terug: evaluatie van de interagerende effecten op de bodem, strooisellaag, regenwormen- en kruidlaaggemeenschap

Illustration on the cover:
Front: deciduous forest
Back: spruce plantation (photograph GertArijs.be)

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Contents

Summary

Forests and forest management in western Europe have changed during the last two centuries. The proportion of coniferous forest has increased since the mid-19th century. As the species composition of the forest overstorey has an impact on the chemical, physical and biological characteristics of the soil, the conversions to coniferous forest may have a strong impact on the forest ecosystem and its functioning. More recently, reconverting secondary non-native coniferous plantations to more natural deciduous forests has become an important topic in forest management. The general aim of this thesis was to quantify the change trajectories of several abiotic and biotic variables after conversion from deciduous forest to Norway spruce (*Picea abies*, further called spruce) plantations and back.

The observational studies in this thesis were performed in ancient deciduous woodlands mainly consisting of *Quercus* spp., *Fagus sylvatica* and *Carpinus betulus* in the Gaume region (southern Belgium). In the first observational study, we resurveyed 43 vegetation plots to see how the herbaceous understorey community of the deciduous stands has changed over the last fifty years. The species pool became more than 10 % smaller on the moderately acidic sites and nearly 30 % smaller on the acidic sites. At the moderately acidic sites, plot species richness declined by 20 %, and the community composition shifted towards a higher frequency and abundance of species requiring less light and more nutrients. The lower management intensity, the abandonment of the coppice with standards management and the increased acidifying deposition were the main drivers of temporal changes in the understorey diversity and composition in the studied deciduous forests.

In the second observatory study, we described the changes in the forest floor, soil variables concerning acidification, earthworm community and understorey vegetation after conversion from deciduous stands to spruce plantations. Spruce plantations were established 35–50 years ago in the matrix of deciduous woodlands. We examined 40 paired plots in spruce plantations and adjacent deciduous forests along a gradient of clay content (as proxy of soil buffering capacity). Spruce is generally considered to be a tree species with strong effects on the forest ecosystem and the impact of conversion on the

studied forests was indeed large. The mean forest floor mass under spruce was three times higher compared to the deciduous stands. The mean pH in the topsoil layer was 0.5 pH units lower under spruce. Significant differences between the earthworm communities under deciduous and spruce trees were observed as well: the earthworm biomass was five times lower under spruce trees and there were less epigeic earthworms and nearly no endogeic earthworms present. The species richness per plot and the species pool of the herbaceous understorey layer did not change after conversion. However, conversion to spruce caused a shift in the understorey species composition towards a higher frequency and abundance of more acid-tolerant and light-demanding species.

In our study area there are both well buffered and moderately acidic soils in the cation exchange buffer range and acidic soils near or in the aluminium buffer range. A second aim of this thesis was to get a better insight in the rates of changes of the measured variables along a gradient of soil buffering capacity. The soil clay content, ranging from 3 to 34 %, was used as a proxy for the natural gradient in soil buffering capacity, independent of the vegetation. The temporal changes in understorey vegetation composition and diversity were larger at the less acid soils. The changes in soil variables, earthworm biomass and the average Ellenberg indicator values for soil reaction of the understorey community between deciduous and spruce forests differ along this gradient with larger changes occurring on the better buffered soils. The decrease in pH and earthworm biomass was several times higher on the better buffered soils.

In the first experimental study, we determined the species-specific effects of light availability and chemical soil characteristics – two important ecological factors that are known to change as a consequence of conversion – on the vegetative and regenerative performance of five characteristic herbaceous forest understorey plants and on the germination rates of two of them. Topsoil samples from both spruce and deciduous stands with two levels of soil acidity were used in a common garden experiment. Additionally, three different light regimes were applied, i.e., ‘light deciduous’, ‘dark deciduous’ (extra light reduction during summer) and ‘evergreen’ (light reduction during winter). The light regime was an important factor affecting both the vegetative and regenerative performance of the understorey species: compared to light deciduous light regime, *Anemone nemorosa* had a significantly lower performance under the evergreen light regime, *Convallaria majalis* under dark deciduous and *Luzula luzuloides* and *Galium*

odoratum under both light regimes. The vegetative performance was lower in soil from acidic sites for the acid-sensitive species *G. odoratum* and *Primula elatior*. Differences in the soil sampled under deciduous or spruce stands had no effect on the vegetative nor regenerative performance of these species, but lowered the germination of the two studied species in the germination experiment, *L. luzuloides* and *P. elatior*.

Reconverting the secondary non-native coniferous plantations into native deciduous forests with the emphasis on biodiversity and close-to-nature forest management became a main aim of sustainable forestry, but the restoration possibilities of these acidified ecosystems are unclear. In the last experimental study, we assessed the effects of two opposite land cover changes (a conversion pathway from deciduous to spruce, and a restoration pathway from spruce to deciduous) on the forest floor, soil acidification and earthworm community. We exchanged soil monoliths from the deciduous to spruce stands (conversion pathway) and vice-versa for the restoration pathway and applied two additional treatments: +/- liming and +/- earthworm. Two and a half year after starting the experiment, the forest floor mass and the earthworm biomass, respectively, increased and decreased significantly under spruce cover. In the restoration pathway, the forest floor mass was significantly lowered and soil pH significantly increased under deciduous tree cover. The earthworm biomass under deciduous tree cover was twice as large as under spruce tree cover. In both the conversion and the restoration pathway, lime addition caused an increase of pH up to 2 pH units while the earthworm treatment had no effect.

Our results provide insights into the effects of conversion from deciduous forests to spruce plantations. The deciduous forests were in a mesotrophic state with a mull-like humus type, soils in the exchange buffer range and with endogeic earthworm species present. Spruce is generally considered to be a tree species with strong ecological effects and therefore the conversion had a large impact on the forest ecosystem, especially on the well buffered soils. After conversion to spruce, the ecosystem shifted towards an oligotrophic state with moder/mor-like humus types, soils in the aluminium buffer range and without endogeic earthworms.

Samenvatting

Bossen en hun beheer in West-Europa zijn sterk veranderd gedurende de laatste twee eeuwen. Het aandeel van fijnspar is gestegen sinds het midden van de 19de eeuw. Doordat de boomsoortensamenstelling een impact heeft op de chemische, fysische en biologische kenmerken van de bodem kunnen we verwachten dat de omvorming naar fijnsparaanplantingen een grote impact kan hebben op het bosecosysteem en zijn functioneren. Recenter werd de omvorming van de standplaatsvreemde fijnsparbestanden naar meer natuurlijke gemengde loofbossen een belangrijk topic in het bosbeheer. Het doel van deze thesis was om de veranderingen van verschillende abiotische en biotische variabelen na de omvorming van gemengd loofbos naar fijnsparaanplantingen en terug te onderzoeken.

De observationele studies van deze doctoraatsthesis werden uitgevoerd in oude loofbossen, met als voornaamste boomsoorten *Quercus* spp., *Fagus sylvatica* en *Carpinus betulus*, in de Gaumestreek (zuiden van België). In de eerste observationele studie hebben we 43 vegetatieopnames heruitgevoerd om te zien hoe de kruidlaag van de loofbossen veranderde gedurende de laatste 50 jaar. De soortenpool werd meer dan 10 % kleiner op de matig zure locaties en meer dan 30 % kleiner op de zure locaties. Op de matig zure locaties daalde de soortenrijkdom in een plot met 20 % en veranderde de soortensamenstelling van de kruidlaag in de richting van minder lichtminnende en meer voedselrijke soorten. De lagere beheerintensiteit, het stopzetten van het hakhoutbeheer en de stijging van de verzurende depositie waren de belangrijkste oorzaken van de veranderingen doorheen de jaren in de kruidlaagdiversiteit en samenstelling in de bestudeerde bossen.

In de tweede observationele studie hebben we de veranderingen na omvorming van loofbos naar fijnsparaanplantingen in de strooisellaag, bodemvariabelen, regenwormengemeenschap en de kruidlaag beschreven. In de matrix van loofbos werden 35-50 jaar geleden eilandjes fijnspar aangeplant. We onderzochten 40 gepaarde plots in de fijnsparbestanden en de aangrenzende loofbestanden langsheen een gradiënt van kleipercentage in de bodem (als proxy van het zuurbufferend vermogen van de bodem). Algemeen wordt aangenomen dat fijnspar een boomsoort is met een sterke ecologische

impact op het bosecosysteem, en dat was ook zo in deze studie. De gemiddelde massa van de strooisellaag onder fijnspar was drie keer zo groot dan onder loofbos. De gemiddelde pH in de bovenste bodemlaag was 0.5 pH eenheid lager onder fijnspar. Ook voor de regenwormengemeenschap zagen we significante verschillen tussen loof en fijnsparbestanden: de regenwormenbiomassa was vijf keer kleiner onder fijnspar en er kwamen minder epigeïsche regenwormen en bijna geen endogeïsche regenwormen in voor. De soortenrijkdom per plot en de soortenpool van de kruidlaag veranderde niet na omvorming. Echter, de omvorming naar fijnspar veroorzaakte wel een verandering in de soortensamenstelling naar een hoger aantal en bedekking van meer lichtminnende en zuurtolerante soorten.

In ons studiegebied kwamen zowel goed gebufferde bodems in het uitwisselingsbufferbereik voor als minder goed gebufferde bodems die zich bevinden op de rand van of in het aluminiumbufferbereik. Een tweede doel van deze thesis was dan ook om een beter inzicht te krijgen in de mate van verandering van de gemeten variabelen langsheen de gradiënt van zuurbufferend vermogen van de bodem. Het aandeel klei in de bodem varieerde van 3 tot 34 % en werd gebruikt als proxy van de natuurlijke gradiënt in zuurbufferend vermogen van de bodem. De temporele veranderingen in de soortensamenstelling en diversiteit in de kruidlaag waren groter op de matig zure locaties. De veranderingen in bodemvariabelen, regenwormenbiomassa en de gemiddelde Ellenberg indicatorwaarden voor de zuurtegraad van de kruidlaag tussen de loof- en naaldbestanden verschillen langsheen de gradiënt met grotere veranderingen op de beter gebufferde locaties. De daling van de pH en de regenwormenbiomassa na omvorming naar fijnspar was verschillende keren groter op de beter gebufferde bodems.

In de eerste experimentele studie bestudeerden we de soort-specifieke effecten van lichtbeschikbaarheid en chemische kenmerken van de bodem – twee belangrijke ecologische factoren waarvan geweten is dat ze veranderen na omvorming – op de vegetatieve en de regeneratieve kenmerken van vijf kruidlaagsoorten en op de kieming van twee van die soorten. Voor dit experiment werd de bovenste bodemlaag uit verschillende loof- en fijnsparbestanden gebruikt in een experiment op één locatie. Daarbij pasten we nog drie verschillende lichtregimes toe: 'licht loof', 'donker loof' (extra schaduw in de zomer) en 'altijd groen' (extra schaduw in de winter). Het lichtregime had een grote invloed op zowel de vegetatieve als de regeneratieve performantie van de kruidlaagsoorten: vergeleken met het ‘licht loof’ regime had *Anemone nemorosa* een significant lagere performantie onder het ‘altijd groen’ regime, *Convallaria majalis* onder ‘donker loof’ en *Luzula luzuloides* en *Galium odoratum* onder beide lichtregimes. De vegetatieve performantie was lager in bodems van zure locaties voor de zuurgevoelige soorten, *G. odoratum* en *Primula elatior.* Het maakte geen verschil voor de vegetatieve en regeneratieve performantie van de soorten of de bodem uit een loof- of fijnsparbestand kwam, maar bodem uit fijnsparbestanden zorgde wel voor een lagere kieming bij de twee soorten uit het kiemexperiment, *L. luzuloides* en *P. elatior.*

Omvorming van de fijnsparbestanden naar meer natuurlijke gemengde loofbossen werd een belangrijk doel in het duurzaam bosbeheer, maar de herstelpotenties van de verzuurde systemen zijn nog onduidelijk. In het laatste experiment hebben we de effecten van omvorming loofbos naar fijnspar en het herstel terug naar loof op de strooisellaag, de bodemvariabelen en de regenwormengemeenschap bestudeerd. Hiervoor werd bodem uitgewisseld tussen loof en fijnspar (omvorming) en omgekeerd (herstel) en werd twee extra behandelingen toegepast: +/- bekalking en +/- regenwormen. Twee en een half jaar na de start van het experiment was het gewicht van de strooisellaag gestegen en de regenwormenbiomassa gedaald onder fijnspar. Bij het herstel daalde het gewicht van de strooisellaag significant en steeg de bodempH onder loof. De regenwormenbiomassa onder loofbos was dubbel zo groot als onder fijnspar. In zowel omvorming als herstel zorgde bekalking voor een stijging van de pH tot meer dan 2 pH eenheden terwijl de regenwormenbehandeling geen effect had.

Onze resultaten geven inzichten in de effecten van omvorming van loofbos naar fijnsparaanplantingen. De loofbossen waren in een mesotrofe toestand met mull-humustypes, een bodem in het uitwisselingsbufferbereik en met endogeïsche regenwormen aanwezig. Algemeen wordt aangenomen dat fijnspar een grote invloed heeft op het bosecosysteem waardoor de effecten van de omvormingen ook groot waren, vooral op de beter gebufferde bodems. Na omvorming naar fijnspar veranderde het ecosysteem naar een oligotrofe toestand met moder/mor humustypes, bodems in het aluminium bufferbereik en zonder endogeïsche regenwormen.

List of abbreviations and symbols

Abbreviations

AIV_L	Average Ellenberg indicator value for light
AIV_R	Average Ellenberg indicator value for soil reaction
AIV_N	Average Ellenberg indicator value for nitrogen
AIV_F	Average Ellenberg indicator value for soil moisture
CEC	Cation exchange capacity
C:N	Carbon:nitrogen ratio
CSR_C	Competitive signature
CSR_S	Stress-tolerant signature
CSR_R	Ruderal signature
DCA	Detrended Correspondence Analysis
F	Fragmentation layer (forest floor)
FFM	Forest floor mass
H	Humus layer (forest floor)
L	Litter layer (forest floor)
LAI	Leaf area index
LQ	Litter quality
RR	Recovery rate
SCA	Shade casting ability index

Definitions

Vegetation layers used in this thesis:

Herbaceous layer	< 1 m, including brambles and ferns
Shrub layer	> 1 m and < 7 m
Tree layer	> 7 m

Mesotrophic	Moderately high nutrient availability
Oligotrophic	Low nutrient availability

1

Introduction

This Phd thesis focuses on the impacts of the conversion from ancient mixed deciduous forests to Norway spruce (*Picea abies*, further called spruce) plantations on the forest floor, soil acidification, earthworm community and understorey vegetation. In the first part of this chapter, we take a look at the large-scale conversions to coniferous plantations performed during the last two centuries, with focus on the conversion from deciduous forests to spruce plantations. In the next part, we will give a brief introduction to tree species effects, and in the last part, we give an overview of the aims and chapters of this thesis.

1.1 Forest conversion

Forests in Europe are since long influenced by man (Table 1.1). At the end of the 18th century, after a long period of overutilisation and devastation of Central European forests through e.g. forest clearing, intense wood harvesting, litter extraction and forest pasture, the wood demand increased even further. In order to meet the increasing wood demand, large forest areas were afforested or converted into highly productive forests. Forest conversion is the sylvicultural process of changing the stand composition and structure.

From the 19th century onwards, the tree species composition of many forests in Western and Central Europe has been changed. Large areas of mixed deciduous forest were converted into fast-growing coniferous plantations offering highly-demanded wood products (Klimo *et al.*, 2000; Spiecker *et al.*, 2004). In large parts of Europe, beech, oak and other deciduous tree species were replaced by fast-growing coniferous tree species

Table 1.1. Past and recent changes of forests and forest sites in Central Europe (After Zerbe, 2002; den Ouden *et al.*, 2010b).

Historical period	**Human impact on the forest**	**Woodland changes**
Early historical times	Forest clearing, because woodland is an impediment to settlements	Decrease of woodland area
Middle Ages and early Modern Times	Uncontrolled, more or less excessive use of forest and forest sites	Overutilization and devastation
End of the 18th to the 19th century	Forest stands managed economically to yield large wood volumes	Afforestation and conversion – increase of woodland area
Since the end of the 20th century	Creating (semi-)natural woodland	Conversion and restoration

such as spruce and *Pinus sylvestris*. Spruce forests now appear far beyond their assumed natural range in Europe (Figure 1.1).

The conversion to coniferous species did increase the wood production, but also has a large impact on the nutrient cycling, biodiversity and resistance against disturbances, e.g. storm, drought and insect or fungus infestations, of the forest (Solberg, 2004; Spiecker *et al.*, 2004). In times of rapid climate change, cultivation of species close to and beyond their distributional limits involves even bigger risks (Bradshaw *et al.*, 2000). Concerns about the environmental impact, potential degradation of soil fertility and sensitivity for disturbances of coniferous plantations have slowed down the extension of spruce plantations. More recently, reconverting these secondary non-native coniferous plantations into more natural deciduous forests became an important aim of sustainable forestry (Mosandl and Küssner, 1999; Spiecker *et al.*, 2004).

1.1.1 Forest conversion in Belgium

Since the 19th century, the forested area in Belgium increased due to afforestation with conifers. In Flanders, this concerns about 33,500 ha of *Pinus sylvestris* and 11,500 ha of *Pinus nigra* var. *laricio* stands on sandy soils (Afdeling Bos & Groen, 2001a). There was no net increase of forest area in Flanders because forest was converted to agriculture land on the well buffered soils (den Ouden *et al.*, 2010b) . In Wallonia, spruce stands were planted after 1850 through the afforestation of mires, bogs, heathland and poor grasslands (“fagnes, bruyères, landes”). Spruce showed high wood production potential, even on poor soils, and produced high quality wood. So, the species was rapidly planted all over the Ardennes.

The forest management in the existing deciduous forests also changed in Wallonia. Many ancient coppice and coppice with standards deciduous forests were transformed to deciduous high forests or to spruce plantations in the 20th century (Noirfalise, 1985). During the 1980s, the area covered by spruce reached a maximum of nearly 200,000 ha, i.e. 37 % of the forested area (545,000 ha) in Wallonia. The growing concerns about the environmental impact of spruce plantations slowed down the planting of spruce in Wallonia, which has resulted in a reduction of the area covered by spruce stands. The area of young spruce stands (less than 20 years old) particularly decreased.

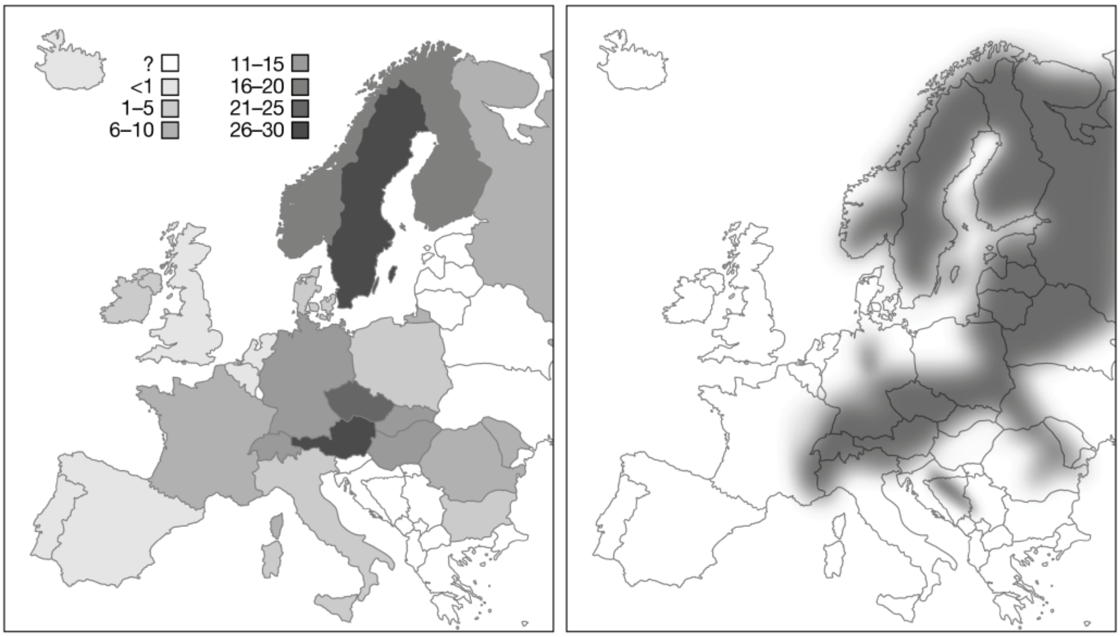


Figure 1.1. Actual distribution (left, percentage of the forest area covered by spruce) and natural range (right) of spruce in Europe (From Klimo *et al*., 2000).

1.2 Tree species effects

The overstorey vegetation, soil chemistry and soil organisms in forests interact closely and can be viewed as co-developing components of terrestrial ecosystems (Zinke, 1962; Van Breemen, 1993; Reich *et al.*, 2005). The species identity and composition of the overstorey can have a large impact on the forest ecosystem. Below, we discuss the most important and best-studied drivers of tree species effects, namely differences in litter quality and quantity, canopy interception and root growth. In the next part, we focus on tree species effects on the forest floor, several abiotic and biotic soil characteristics and the understorey vegetation, i.e. the variables studied in this thesis.

1.2.1 Drivers of tree species effects

1.2.1.1 Litter quality and quantity

Litter quality is tree species specific. The decomposition rate of leaf litter is largely determined by the litter quality; litter with good quality having a high decomposition rate. The species-specific variation in litter decomposition is often related to physico-chemical characteristics of the leaf, e.g. morphology, hardness and chemical composition (Berg, 1986; Augusto *et al.*, 2002; Reich *et al.*, 2005; Hobbie *et al.*, 2006; Vesterdal *et al.*, 2008; Vivanco and Austin, 2008). Studies have identified various variables related to the chemical composition of leaf litter that determine the decomposition rate. The variables that are most often reported are the ratio of carbon to nitrogen (C:N), the lignin concentration, the lignin:N ratio and the calcium concentration. Augusto *et al.* (2002) refer in their review to several articles that show big differences in nutrient concentrations of the leaf litter, especially between deciduous and coniferous tree species. Litter nutrient concentrations were 10 to 50 % higher for N and phosphorus (P) and 100 to 400 % higher for calcium (Ca), magnesium (Mg) and potassium (K) in deciduous tree species than in conifers, but there are also big differences within both species groups. Calcium concentrations are especially important when earthworms are present; high leaf litter calcium concentrations are associated with increased earthworm abundance and activity (Reich *et al.*, 2005; De Schrijver *et al.*, 2012). Low litter quality, and thus slowly decomposing litter, is generally caused by a low calcium concentration, a high C:N ratio and a high lignin, cellulose and hemicellulose concentration or lignin:N ratio.

In temperate forest, the litter quantity is mainly determined by stand structure, e.g. influenced by forest management, but litter quantity will also differ between tree species (Augusto *et al.*, 2002).

1.2.1.2 Canopy interception and filtering capacity

The interception of atmospheric deposition differs between tree species and is strongly influenced by canopy structure, e.g. leaf area index and aerodynamic roughness (Binkley, 1995; Augusto *et al.*, 2002). The interception by coniferous species is often higher than for deciduous species (Nordstrøm and Rundgren, 1974; Ulrich, 1983a; Augusto *et al.*, 2002; Rothe *et al.*, 2002; De Schrijver *et al.*, 2007). During winter, the differences are more pronounced because then, the interception rates of conifers, due to their evergreen character, will be higher than those of deciduous tree species. The usually higher LAI of conifers also causes a generally higher interception of potentially acidifying elements, e.g. N and sulphur (S). For instance, in several studies, Augusto *et al.* (2002) found deposition under spruce to be nearly three times higher than in open area, compared to at most twice as high for oak *Quercus* spp., *Fagus sylvatica* and *Carpinus betulus*.

The light transmission through the crown also differs between tree species. The seasonal changes in light conditions will be different in evergreen stands compared to deciduous stands, but also in summer, the variation in light transmission between tree species can be large (e.g. Canham *et al.*, 1994; Ellenberg and Leuschner, 2010). Forest management measures like thinning can also have a highly significant influence on the light transmission.

1.2.1.3 Root growth and nitrogen fixation

Species-specific effects on the forest ecosystem via roots are less studied. The amount of acid exudated by tree roots varies between species and will influence soil acidification. For example, the rhizosphere of spruce is more acid than the rhizosphere of several other European species such as oak and beech (Calvaruso *et al.*, 2011). Trees also have a physical impact on the soil through their roots (Augusto *et al.*, 2002). Nitrogen fixation by tree species can enhance soil acidification through proton (H^+) production as well as by the leaching of cations associated with mobile nitrate (NO_3^-) (Van Miegroet and Cole, 1984; De Schrijver *et al.*, 2012).

1.2.2 Consequences of tree species effects

1.2.2.1 Tree species effects on the forest floor

Tree species affect the forest floor via the quality and the quantity of their leaf litter. The litter quality of tree species largely drives the humus type in case of comparable climatologic and soil characteristics and management history. A very convenient way to divide tree species into groups with a different impact on the forest ecosystem is the distinction between moder/mor-forming and mull-forming tree species (Figure 1.2)

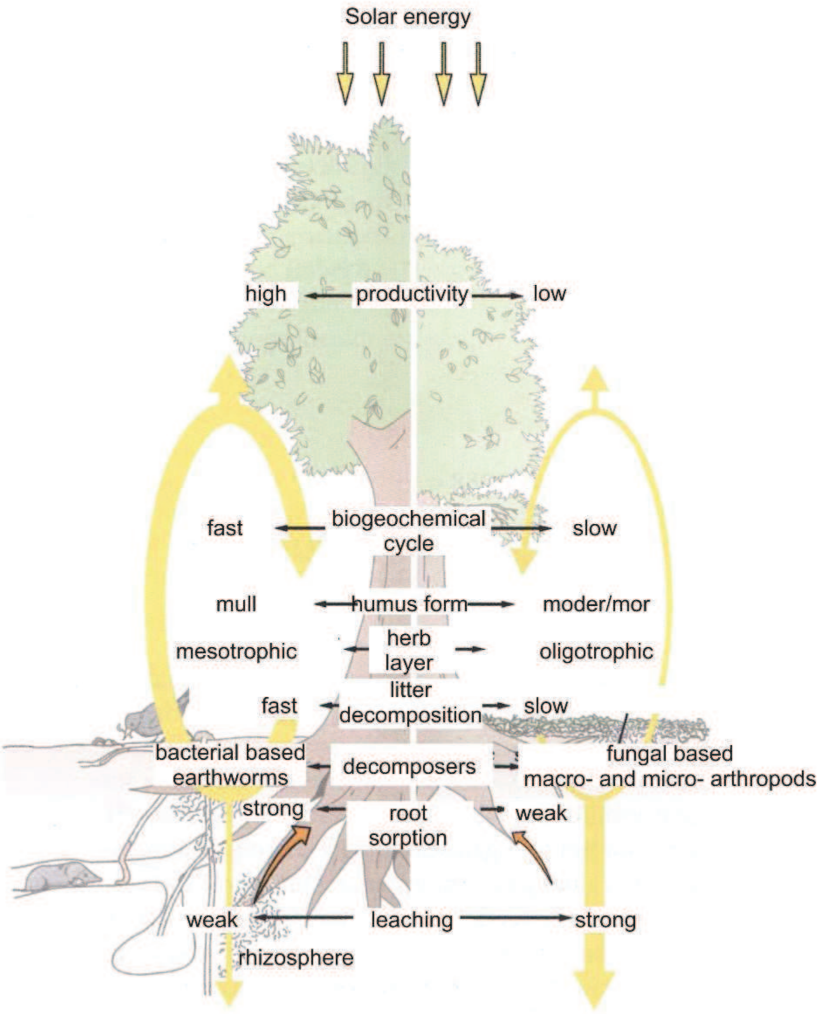


Figure 1.2. Characteristics associated with mull-forming and moder/mor-forming tree species. Mull-forming tree species (left) typically have fast-decomposing leaf litter; bacteria and earthworms are present in the decomposer soil community; nutrient cycling is fast; leaching is weak; and the herbaceous understorey community is mesotrophic. (After Toutain, 1981; Muys and Granval, 1991; den Ouden *et al.* 2010a)

(Toutain, 1981; Muys and Granval, 1991; Van Calster, 2008). Mull, moder and mor humus types are the three main humus types according to e.g. Jabiol *et al.* (1995) (Table 1.2, Figure 1.3). Mull is characterised by an intense mixing of organic matter with mineral soil. Soil fauna including earthworms and bacteria cause rapid organic matter decomposition and nutrient cycling, which results in a crumbly and nutrient-rich organo-mineral horizon. Moder is characterised by a less rapid transformation of litter by litter-dwelling animals and fungi, which results in the accumulation of organic humus at or near the soil surface. Mor is characterised by the slow transformation and accumulation of undecayed plant debris, with a sharp, clearly defined transition from the humus to the mineral soil. The decomposition is relatively slow and predominantly done by fungi.

Mull-forming tree species have a good litter quality and therefore fast-decomposing leaf litter. Moder/mor-forming tree species have slowly-decomposing leaf litter, which usually results in a thicker fragmentation (F) layer and an accumulation of organic matter in the humus (H) layer. Species with high leaf litter calcium concentrations such as *Tilia* spp. and *Acer* spp. show rapid litter decomposition and high earthworm abundance (Reich *et al.*, 2005; Hobbie *et al.*, 2006). Species such as *Picea* spp. and *Pinus* spp. generally have

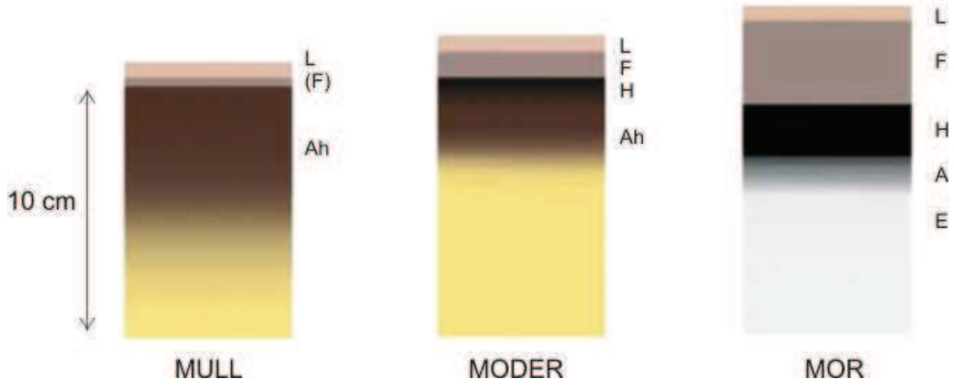


Figure 1.3. Structure of a typical mull, moder and mor topsoil profile (From den Ouden *et al.*, 2010a). L (litter layer): Horizon characterized by the accumulation of mainly leaves/needles, twigs and woody materials. F (fragmentation layer): Horizon characterized by the accumulation of partly decomposed (i.e. fragmented, bleached, spotted, skeletonized) litter, mainly from transformed leaves/needles, twigs and woody materials, but without any entire plant organ. H (humus layer): Horizon characterized by an accumulation of zoogenically transformed material, i.e. black, grey-brown, brown, reddish-brown well-decomposed litter, mainly comprised of aged animal droppings. A organo-minaral horizons. E eluviated mineral soil.

Table 1.2. Characteristics of terrestrial humus forms (after Jabiol *et al.*, 1995; Zanella *et al.*, 2011).

		Mull	**Moder**	**Mor**
Forest floor	litter layer	possible	present	present
	fragmentation layer	possible, zoogenically transformed	present, zoogenically transformed	present even if sometimes discontinuous; not zoogenically transformed
	humus layer	absent	present, active, sometimes discontinuous	present, inactive or partially active
Transition forest floor - minaral soil		very sharp (< 3 mm) discontinuity	not sharp (≥ 5 mm)	very sharp (< 3 mm)
Organo-mineral horizon - A		biomacro or biomeso	biomicro or single grain or massive	absent (= E) or present, if present: not zoogenic or discontinuously biomicro.
Horizon of dominant faunal activity		A (anecic and endogeic earthworms)	F (feeding) H (accumul. droppings)	H (weak or traces of old activity)
Earthworms *	organic horizons	epigeic and anecic	epigeic	epigeic rarely present or absent
	organo-minaral horizon	endogeic and anecic	abscent	Absent

* description of earthworm ecological groups in Figure 1.5.

very poor litter and accumulate a substantial forest floor mass (Hobbie *et al.*, 2006). The forest floor mass under *Picea abies* could be up to twice that of hardwood species such as *Fagus sylvatica* (Augusto *et al.*, 2002). Especially in the soil pH-range between 3.5 and 4.6, tree species are decisive in determining the humus type. *Tilia* spp., *Acer* spp. and *Fraxinus excelsior* could have mull humus types until a pH of 3.5 to 4, while for e.g. *Quercus* spp. and *Fagus sylvatica* the turning point to moder/mor humus is already at about pH 4.5 (Rackham, 1980).

1.2.2.2 Tree species effects on soil characteristics and soil acidification

Ecologists and pedologists have long recognized that vegetation can influence soil properties (Jenny, 1941; Zinke, 1962), and recent evidence suggests that plants can change soils relatively rapidly (Wedin and Tilman, 1990; Muys *et al.*, 1992; Berendse 1998; Binkley and Giardina, 1998).

Soil acidification is the accumulation of hydrogen ions, also called protons (H^+), in the soil. A soil acidifies when its acid-neutralising capacity decreases and the proportion of cations such as K^+, Ca^{2+} and Mg^{2+} on the clay-humus-exchange complex decreases at the benefit of aluminium (Al^{3+}) and iron (Fe^{2+}) (de Vries and Breeuwsma, 1985). Soils are buffered against acidification by a series of buffer reactions (Box 1). Acidification of the forest soil is a natural process, which is, however, accelerated by human activity.

Tree species can have a high impact on soil acidification. De Schrijver *et al.* (2012) proposed a conceptual model to explain tree species effects on soil acidification (Figure 1.4). The forest floor, and thus the leaf litter quality, has an important effect on soil pH and exchangeable cation concentrations. In the case of moder- and mor-forming tree species, a thick forest floor is built up, which can cause soil acidification through the production of organic acids as intermediate decomposition products (de Vries and Breeuwsma, 1985) and by causing a time delay in the return of buffering cations to the soil where they are taken up by tree roots in exchange for protons (Nilsson *et al.*, 1982). Tree species with high filtering capacity cause a higher potentially acidifying input through atmospheric deposition, which will result in a higher soil acidification rate. Root uptake of nutrients and the amounts of acid exudated by tree roots vary between species and influence to a lesser extent the soil variables concerning acidification. Based on several studies, Augusto *et al.* (2002) ranked tree species in order of decreasing acidifying ability as follows: (1) *Picea abies*, *Picea sitchensis*, *Pinus sylvestris* > (2) *Abies alba*, *Pseudotsuga menziesii* > (3) *Betula pendula*, *Fagus sylvatica*, *Quercus petraea*, *Quercus robur* > (4) *Acer platanoides*, *Carpinus betulus*, *Fraxinus excelsior*, *Tilia cordata*.

Tree species also influence the physical properties of the soil (e.g. Binkley and Giardina, 1998; Augusto *et al.*, 2002). Light transmission through the tree canopy differs between tree species and influences the soil temperature. Differences in canopy interception and transpiration between tree species cause differences in soil moisture (Binkley, 1995). Soil aeration may be altered by root activity, e.g. through oxygen consumption and changes in soil porosity and perhaps structure.

1.2.2.3 Tree species effects on the earthworm community

The response of the earthworm community to tree species is complex. On the one hand, earthworms are sensitive to changes in environmental conditions. On the other hand, earthworm species also influence the chemical soil conditions through their role in litter

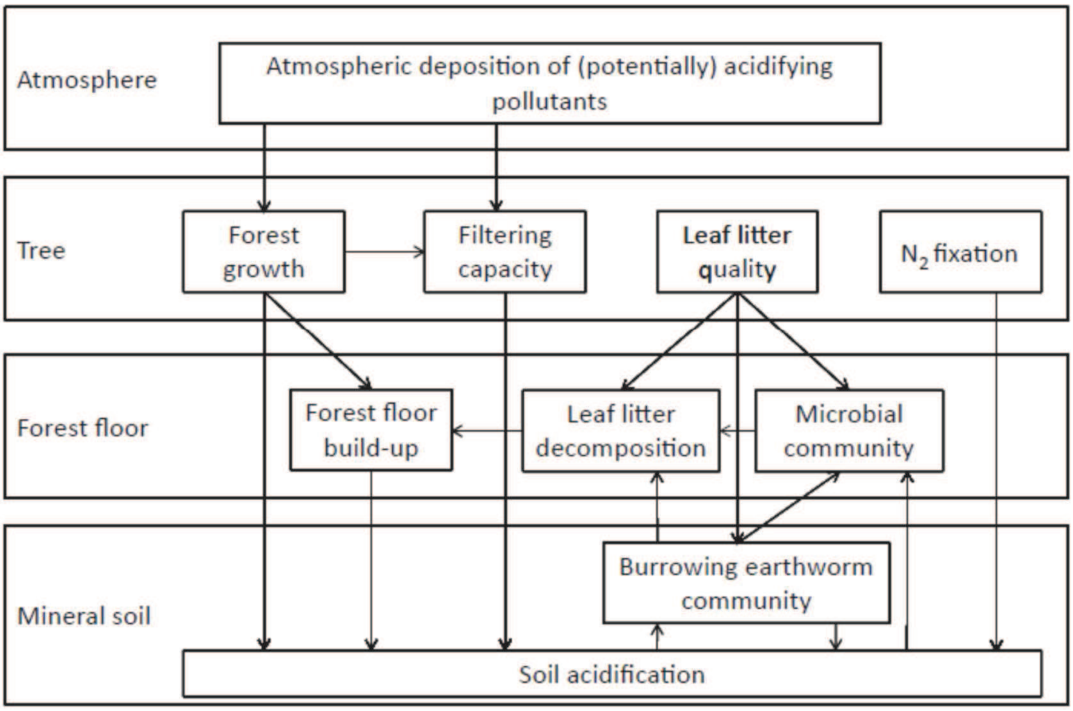


Figure 1.4. Conceptual model of tree species-related causes of soil acidification in temperate forests (from De Schrijver et al., 2012)

decomposition and mixing. When earthworms are present in the soil, they typically play a major role in the translocation and decomposition of leaf litter (Lavelle, 1997; Edwards, 1998). High species diversity and abundance of earthworms lead to increased organic matter turnover rates (Ponge *et al.*, 1999). Several earthworm species show bioturbation activity: they are incorporating organic matter from the forest floor into the deeper soils. Earthworms, especially endogeic (shallow soil dwellers, Figure 1.5) and anecic species (deep-burrowers that feed at the surface on litter which they pull into their vertical burrows), are important in sustaining mull humus quality (Muys and Lust, 1992; Edwards, 2004).

Tree species are an important determinant of the forest floor and therefore, soil conditions can influence the earthworm community significantly. Earthworm species are sensitive to acidification: soil acidification is related to decreased exchangeable base cations concentrations and increased potentially toxic aluminium concentrations, both harmful for earthworm communities (e.g. Muys, 1989; van Gestel and Hoogerwerf, 2001; Reich *et al.*, 2005). Tree species influence the earthworm community by their litter quality: leaf litter rich in calcium exhibits fast litter decomposition and is related with high earthworm abundance; species with low leaf litter calcium concentrations are associated with lower

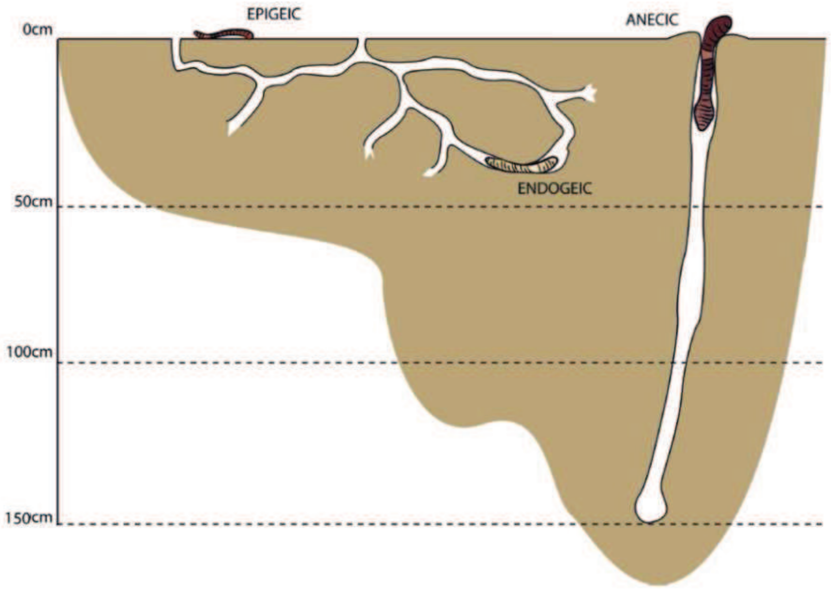


Figure 1.5. Three ecological earthworm groups: epigeic (small litter dwellers, make almost no burrows), endogeic (shallow soil dwellers) and anecic (deep-burrowers that feed at the surface on litter which they pull into their vertical burrows) (from Schelfhout, 2010).

decomposition rates and low earthworm abundances (Reich *et al.*, 2005; Hobbie *et al.*, 2006; De Schrijver *et al.*, 2012). Earthworms are also very sensitive to dehydration (e.g. Nordstrøm and Rundgren, 1974; Grossi and Brun, 1997; Wever *et al.*, 2001; Eggleton *et al.*, 2009), and the lower humidity of the forest floor and soil under some tree species can adversely affect the earthworm community.

1.2.2.4 Tree species effects on the understorey vegetation

The understorey diversity and composition is profoundly influenced by the species composition of the tree canopy (Hunter, 1999; Gilliam 2007; Barbier *et al.*, 2008) and by site conditions such as soil and microclimate characteristics (Augusto *et al.*, 2003). The overstorey directly affects the understorey through its impact on light availability (Härdtle *et al.*, 2003; Tinya *et al.*, 2009) and by competition for water and nutrients (Small and McCarthy, 2005; Van Calster *et al.*, 2008). Trees also have indirect effects on the understorey, especially through the quality and quantity of their litter, which influences the forest floor characteristics, the chemical soil conditions (Augusto *et al.*, 2002; Mölder *et al.*, 2008; Thomaes *et al.*, 2011), the soil fauna (Reich *et al.*, 2005) and, hence, the recruitment and growing conditions of the vegetation (Sydes and Grime, 1981; Verheyen *et al.*, 2003b; Baeten *et al.*, 2009).

1.3 Aims and outline of the thesis

During the last three decades, politicians and policy makers became more and more concerned about biodiversity loss. Now, it is clear that biodiversity loss will have a high impact on the functioning of ecosystems and their ability to provide goods and services to society. Therefore, biological conservation ranks high on the policy agendas (e.g., Millennium Ecosystem Assessment, 2005). In forestry, the interest in biodiversity conservation has also increased, and the conservation of biodiversity has become an important goal of multifunctional and sustainable forest management (e.g., Ministerial Conference of the Protection of Forest in Europe, 1993). Tree species changes, often to exotic species, lead to notable modifications of the whole forest ecosystem. The conversion to a non-native tree species can have a large influence on the species diversity of several ecological groups in the forest. Next to the loss of biodiversity, soil acidification due to tree species changes can cause nutrient depletion, lower productivity and, on the longer term, loss of ecosystem stability. Thus, it is important to investigate the short- and long-term impacts of forest conversion on soil fertility and biological diversity.

More recently, reconverting secondary non-native coniferous plantations to more natural deciduous forests has become an important topic in forest management (Mosandl and Küssner, 1999; Spiecker *et al.*, 2004). The **primary aim** of this thesis was to quantify the effects of forest conversion to spruce plantations and back on several biotic and abiotic variables of the forest ecosystem.

Different soil buffering mechanisms (Box 1) could cause differences in the changes of the soil acidity along the gradient of buffer capacity as response to the increased acidifying input after conversion to spruce. Therefore, we expect that the changes in the soil acidity, but also in other response variables measured in this thesis could differ in size along the gradient of soil buffering capacity. The **secondary aim** was to get a better insight into how these processes are triggered and whether the mechanisms are the same along a gradient of soil buffering capacity. This knowledge about this aim is rather limited and the possibility to study several variables along a gradient in a relatively small area makes this study unique.

We focus on the conversion from mixed deciduous forest to spruce plantations and the effects on the forest floor, soil acidification, the earthworm community and the understorey vegetation. The thesis is divided into an observational and an experimental part. Figure 1.6 gives an overview of the variables studied in each chapter.

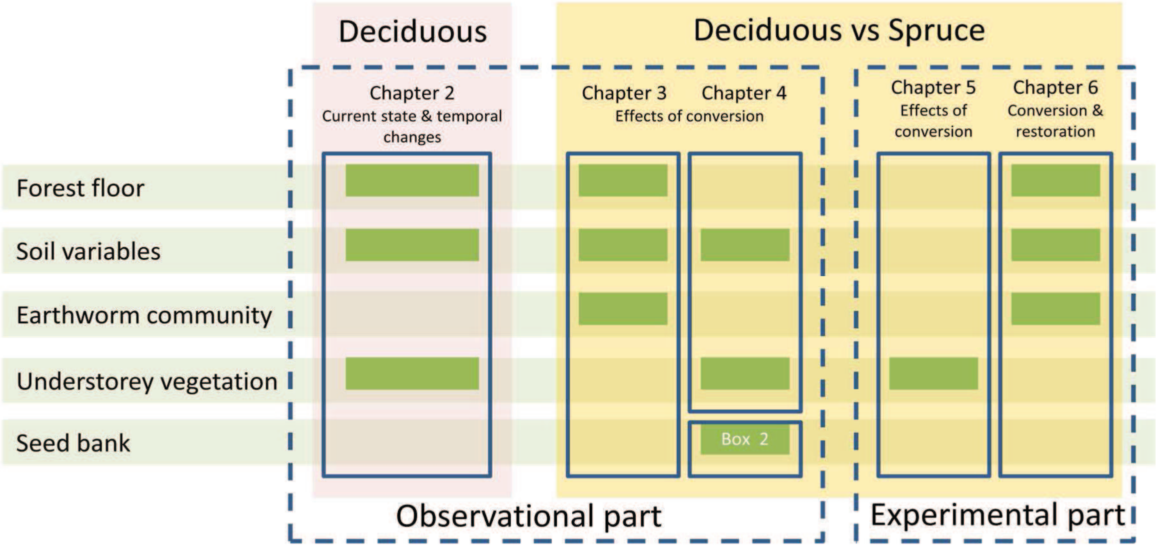


Figure 1.6. Schematic outline of the five chapters in this thesis. The left part of the scheme shows the response variables measured in this thesis. The green rectangles showed the response variables measured in each chapter.

1.3.1 Observational part

The study area (49° 37’ N, 5° 33’ E; approximately 10 km²) is part of a large forest complex in the south of Belgium (Gaume region, Figure 1.7). The soil parent material is sandstone with variable lime content (Ghysel *et al.*, 2002; Belanger, 2006). The soil type is Lamellic Arenosol (Soil atlas of Europe, 2005) and the soil texture varies between sand, loamy sand or sandy loam (Service Public de Wallonie, 2007). The different depths and lime content of the substrate and the different texture classes create a gradient of soil fertility and buffering capacity. Elevation ranges between 250 and 360 m above sea level. The slope is mostly nearly level or gently sloping.

The climate is sub-Atlantic with the mean annual precipitation amounting to 873 mm evenly distributed throughout the year. The mean annual temperature is 8.7°C; the mean temperature during the growing season (April–September) is 13.7°C (Royal Meteorological Institute of Belgium, http://www.kmi.be).

The study area has been forested since at least the oldest full coverage, reliable topography map for the Austrian Low Countries (now Belgium) of 1777 (De Ferraris Map). The deciduous forest matrix is mainly uneven-aged high forest dominated by *Quercus robur* (percentage of total estimated tree canopy cover in the studied deciduous plots in chapter 3 and 4: 27%), *Quercus petraea* (6%), *Fagus sylvatica* (22%) and *Carpinus betulus* (36%). The area is located in two special protection areas from the Natura 2000 Network (‘Bassin supérieur de la Chevratte’ and ‘Vallées de Laclaireau et du Rabais’). Several deciduous forest stands are classified as important habitats to be protected in the EU Habitat Directive (Council Directive 92/43/EEG, 1992). The most abundant forest types in the study area are *Luzulo-Fagetum* beech forests (habitat type 9110) and sub-Atlantic and medio-European oak or oak-hornbeam forests of the *Carpinion betuli* (habitat type 9160). In a smaller area, *Asperulo-Fagetum* beech forests (habitat type 9130) are present. In the region, there is also a significant area of alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion, Alnion incanae*) (habitat type 91E0), but none of the study plots were situated in this forest type.

The study area is ancient forest (forested at least since the Ferraris map, 1777) with a relatively undisturbed understorey layer in equilibrium with the site conditions. The former coppice with standards management system was abandoned in the mid-20th century. Today, the forest is dominated by trees originating from resprouts after the last cut of the coppice wood, together with some remaining standards and younger regeneration. There is a mixture of forest management types, but the management is mostly not intensive. The thinning regime depends on the location; at some sites, only trees of interest are cut, at other sites, real high or low thinnings are performed. Firewood companies are also active in the region.

Between 1950 and 1990, patches of 2 to 15 ha in this deciduous matrix were harvested and replanted with monoculture stands of spruce, which created random islands of coniferous forest within the larger deciduous matrix. The patch locations were mostly randomly chosen in the deciduous forest. As far as we known, the conversion has taken place without large disturbances or intensive soil tillage such as plowing. The spruce stands are mostly managed through high thinning, and their crown closure is lower compared to the deciduous stands.

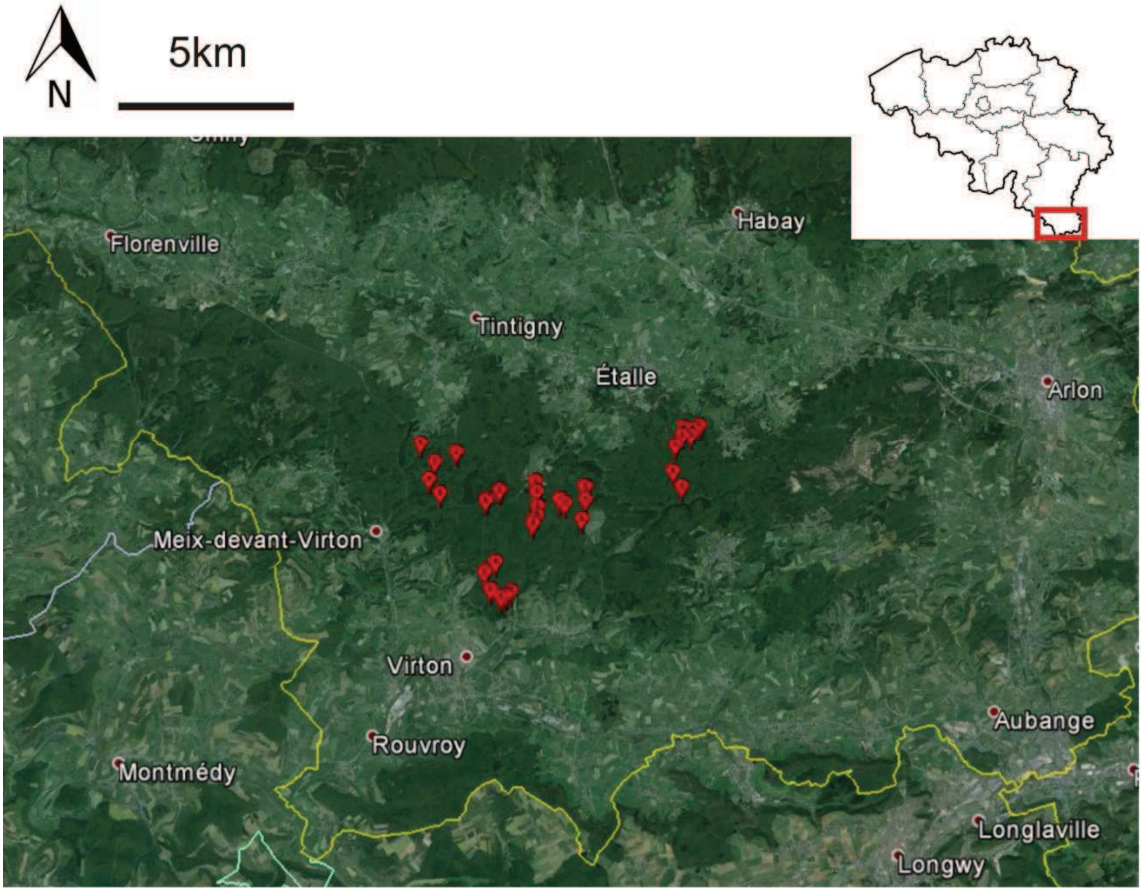


Figure 1.7. Location of the study area and plots (red points) in the Gaume region (map data: Aerodata International Surveys, 2013; DigitalGlobe, 2013; IGN-France 2013, Google, 2013)

The **first specific aim** was to get a better insight into the long-term temporal changes in the herbaceous understorey community of the deciduous stands during the last fifty years (Chapter 2). The management of the deciduous stands also changed during the last decades; the former coppice-with-standards management system was abandoned in the mid-20th century. Today, the forest is dominated by trees that resprouted after the last cut of the coppice together with some remaining standards and younger regeneration. In 2008, we re-inventoried 43 vegetation recordings of 1953-1954 in deciduous forests to study the temporal changes in the herbaceous understorey vegetation.

Spruce is generally considered a tree species with strong negative ecological effects due to its recalcitrant leaf litter, continuous leaf cover and high leaf area index (LAI) (e.g. Ranger and Nys, 1994; Augusto *et al.*, 2002). Therefore, the **second specific aim** of the thesis was to describe the changes in the forest floor, soil variables concerning acidification, the earthworm community, the understorey vegetation and the seed bank after conversion from deciduous stands to spruce plantations (Chapter 3, Chapter 4 & Box 1). To our knowledge, the effect of a specific conversion type (e.g., from mixed deciduous to spruce monoculture) along a relatively long soil gradient has never been studied in a comparable manner. This study is also very suitable to enlarge the knowledge about the relation between all the variables studied in the different chapters.

The study region is characterised by variation in soil buffering capacity, mainly created by differences in soil texture. We used this natural gradient in the chapters 2-5 to fulfil the second aim of this thesis. The trajectories of change after conversion were described and discussed along this gradient.

1.3.2 Experimental part

The experimental part builds further on the results of the observational part and investigates more into detail the effects on the understorey vegetation and possibilities for restoration. The **third specific aim** was to assess the effects of conversion from deciduous stand to spruce plantations on the adult performance and germination of several forest understorey species (Chapter 5). The knowledge about the mechanisms behind the vegetation changes after conversion is limited, and the combination of both observational and experimental research could bring new insights into conversions. We experimentally determined the effects of two important ecological factors that are known to change as a consequence of conversion, i.e. light availability and chemical soil characteristics, on the vegetative and regenerative performance of five characteristic herbaceous forest understorey plant species.

The **fourth specific aim** of this thesis was to assess the effects of restoration of spruce plantations by reconversion to deciduous stands on the soil variables concerning acidification, the forest floor and the earthworm community (Chapter 6). In this experiment, we simulated conversion and restoration by exchanging soil monoliths between deciduous and spruce stands. We also studied whether a lime addition and the absence or presence of earthworms are stimulating or slowing down the conversion or restoration trajectories.

Box 1: Soil buffer ranges and mechanisms

Soil acidification is the build-up of hydrogen cations, also called protons, in the soil. A soil acidifies when its acid neutralising capacity decreases and the proportion of K^+, Ca^{2+} and Mg^{2+} on the clay-humus-exchange complex decreases in advantage of aluminium and iron (de Vries and Breeuwsma, 1985). Soil acidification is caused by a flux in protons and is buffered by a subsequence of buffer reactions. Ulrich and Sumner (1991) determined a subsequence of acid buffer ranges in the soil (Table Box 1.1, Figure Box 1.1). The buffer range pH limits may vary depending on the composition of the buffer substances and on how much the soil state deviates from the chemical equilibrium (De Schrijver, 2007). As long as carbonates are present in the soil, acids are buffered by carbonate dissolution and the pH-H_2O will stay in the range 6.2-8.6. When all carbonate has been consumed, mineral weathering is the only mechanism which can buffer incoming acids and the pH-H_2O drops below 6.2. Mineral weathering is supposed to happen at any pH. Its buffering capacity is generally large and depends on the soil type and to a lesser degree on the forest type (Augusto *et al.*, 2002), but its buffering rate is determined by the weathering rate (Kauppi *et al.*, 1986). The soil pH will further decrease if the acid input is higher than the buffering capacity and comes into the cation exchange buffer range where the excess protons will be buffered by exchangeable bases (Kauppi *et al.*, 1986). The exchange buffering capacity is small and once the base saturation drops below 5-10 % the forest soil enters the aluminium buffer range (prevailing pH-H_2O of 3.8-4.2) where acid is buffered by dissolution of aluminium silicates. At pH values < 3.2, in horizons influenced by infiltrating organic matter with a pH below 3.8, the solubility of iron oxides becomes high enough to reach iron concentrations in the soil solution of ecological significance (De Schrijver, 2007).

Table Box 1.1. Mechanisms of acid buffering for different pH ranges and acid buffer capacity for each of the buffer ranges (after Ulrich, 1987; Ulrich and Sumner, 1991; Malessa, 1995).

Buffer range	pH H_2O range	Base saturation (%)	Buffer reaction	Buffer capacity/ha
Carbonate	8.6 > pH > 6.2	100	$CaCO_3 + H^{+}HCO_3^{-} \rightarrow Ca^{2+} + 2HCO_3^{-}$	150 kmol H+ per % CaCO3
Silicate	pH independent; dominant at 6.2 > pH > 5	100-70	$CaAl_2Si_2 0_8 + 2H_2CO_3 + H_2O \rightarrow Ca^{2+} + 2HCO_3- + Al_2Si_2O_5(OH)_4$	25 kmol H+ per % silicate
Exchange	5 > pH > 4.2	70-5	clay mineral=Ca + 2H+ → H–clay mineral–H + Ca^{2+}	7 kmol H+ per % clay
Aluminium	4.2 > pH > 3.0	5-0	$AlOOH + 3H^{+} \rightarrow Al^{3+} + 2H_2O$	100-150 kmol H+ per % clay
Iron	pH < 3.8	0	$FeOOH + 3H^{+} \rightarrow Fe3^{+} + 2H_2O$	

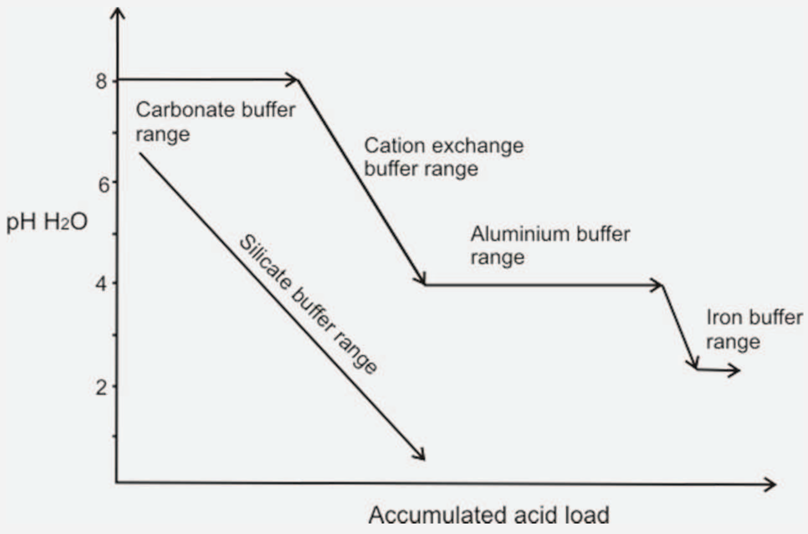
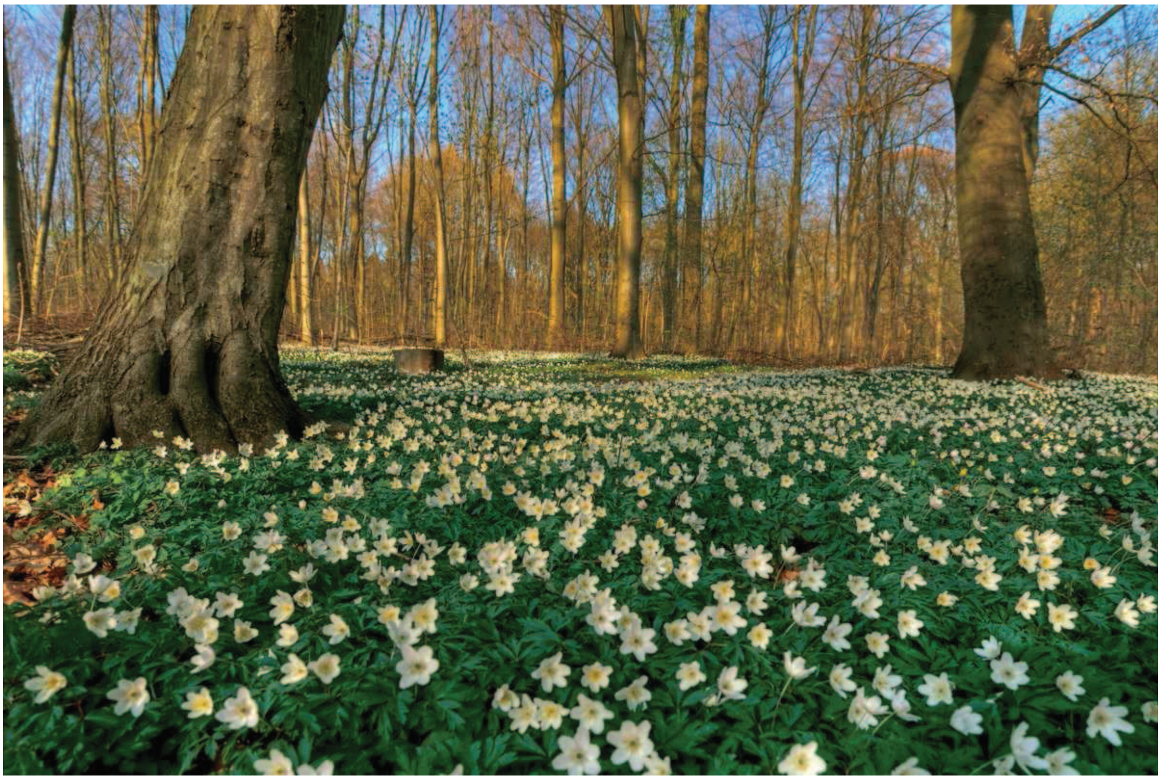


Figure Box 1.1. Buffer ranges and changes in pH during soil acidification (Ulrich and Sumner, 1991; De Schrijver, 2007).



Anemone nemorosa

2

Temporal changes in forest plant communities at different site types

After: Verstraeten G., Baeten L., Van den Boeck T., De Frenne P., Demey A., Tack W., Muys B. & Verheyen K. (2013). Temporal changes in forest plant communities at different site types. Applied Vegetation Science, 16, 237-247.

Abstract

Given the significance of the herbaceous understorey layer for temperate forest ecosystem biodiversity and functioning, it is important to have a thorough understanding of its dynamics in response to environmental change. However, site-specific factors such as soil type may cause differential temporal herb-layer changes within a single study area under comparable external pressure. Surprisingly, relatively few studies have examined herb-layer changes on different site types within the same forest complex. The aim of this study is to determine the temporal differences in herb vegetation and to compare these for two differing site types in the same forest complex.

The study plots were located in ancient broadleaved woodlands traditionally managed as coppice with standards in the South of Belgium, an area with Secondary deposits and a mild temperate climate. In 2008 we re-inventoried 43 vegetation recordings from 1953–1954 in forests on two site types (acidic and moderately acidic forests). First, we described differences in soil pH, litter and overstorey characteristics between the sites. Next, multivariate analysis, Ellenberg indicator values and dominant plant strategies (CSR-signature) were used to determine differences in herb layer diversity and composition between both the two survey times and the two site types. Finally, we analysed temporal differences in individual species abundances and frequencies.

The two site types clearly differed with respect to soil, litter and overstorey characteristics. Temporal changes between the surveys were mostly highly significant on the moderately acidic sites and mostly not significant on the acidic sites. On both site types, the species pool size declined. On the moderately acidic site, also the mean plot species richness declined. From the plant-derived characteristics (Ellenberg indicator values) of the plots, we learned that the light availability decreased and nitrogen availability significantly increased on the moderately acidic sites.

Input of eutrophying deposition and management changes are seen as key drivers of herb layer changes in the study area over the last half century. However, our study suggests that site characteristics such as parent material and soil pH should be considered as well. Together with the key drivers, incorporating differences in site characteristics may improve our understanding of temporal shifts in herb-layer vegetation in response to environmental change.

2.1 Introduction

Despite the small stature, the herbaceous understorey layer is important at the ecosystem level in forests (Gilliam, 2007). Given its significance in e.g. forest ecosystem functioning, it is important to establish a good understanding of the dynamics of the understorey layer. During the last decade, several studies in temperate forests have identified remarkable changes in the herb layer vegetation composition over time (e.g. Van Calster *et al.*, 2008; Baeten *et al.*, 2009a; Verheyen *et al.*, 2012). Usually, those studies focused on the driving factors responsible for changes in the understorey communities. But less well understood is how change trajectories can differ because of variation in geological site characteristics between locations. Here, we investigated how site-specific factors cause differential temporal herb-layer changes within a single study area, i.e. keeping the external drivers constant.

Temporal changes in herb layer communities in temperate ancient forests are often attributed to a combination of different driving factors including (i) the input of potentially acidifying and eutrophying atmospheric deposition (e.g. Thimonier *et al.*, 1992; Lameire *et al.*, 2000; Bernhardt-Römermann *et al.*, 2007), (ii) changes in forest management and intensity (e.g. Van Calster *et al.*, 2007; Baeten *et al.*, 2009a) and (iii) changes in grazing pressure (e.g. Kirby and Thomas, 2000; Rooney and Waller, 2003; Rooney, 2009). Whereas the temporal changes in species composition of the herb layer vegetation are generally significant, their magnitude and direction often vary among studies. This variation is probably due to differences in the nature of the underlying environmental changes and altered disturbance regimes, but may also be related to inherent site differences (Diekmann and Lawesson, 1999; Ellenberg and Leuschner, 2010; Verheyen *et al.*, 2012). Verheyen *et al.* (2012) for instance, analysed decadal vegetation changes of more than 20 studies in lowland forests across western and central Europe. Changes in canopy species composition and cover were important factors explaining the changes in the understorey, but, at the same time, considerable unexplained variation between the studies remained, which may be partly related to differences in site characteristics within and between the studies.

Variations in site characteristics are often related to differences in soil conditions. Soils weathered from alkaline bedrocks have a higher pH and higher concentrations of exchangeable cations in the mineral soil layer than soil weathered from non-alkaline bedrocks (Vestin *et al.*, 2006). Soil texture influences water and nutrient retention (Troeh and Thompson, 2005). Chemical and physical soil properties can profoundly influence the composition of the herb layer (Falkengren-Grerup *et al.*, 1995; Tyler, 1996; Ellenberg and Leuschner, 2010). Therefore, differences in parent material and soil type will be reflected in the composition of the understorey layer of forests. Different vegetation types could have a different succession pathway (Roberts and Gilliam, 1995) and therefore, they could generate a different response on changes of external influences (e.g. management and deposition) during longer time periods. External influences could also interact in a different way with soil characteristics and, hence indirectly influence the vegetation. For example, the sensitivity to acidifying deposition depends on the cation exchange capacity and the amounts of weatherable minerals (Wiklander and Andersson, 1972). To explore variation in understorey shifts under comparable external drivers, we can investigate different site types in the same forest complex. So far, relatively few studies have examined herb layer changes within the same forest complex whereby they specifically looked to differences in temporal changes along a gradient of site types (like substrate properties or chemical soil properties). Lameire *et al*. (2000), for instance, found clearly discernible temporal changes between two site types, but the contrast between the site types was high (alluvial vs. plateau forest). Thimonier *et al*. (1994), on the other hand, found clear differences among sites with an eutrophication signal in the plant community change on moderately acid (mean pH 5.1) as well as on calcareous (mean pH 6.9) site types, but with an acidification signal on the moderately acid soil. Van Calster *et al*. (2007; 2008) looked to shifts in soil and vegetation after conversion from coppice with standards to different types of high forest on two site types, but the effect of conversion type was more pronounced than the site effect. All these studies have shown a different response of the herb layer on different site types in response to the same external driver(s) of change.

Hence, we can expect differences in herb layer changes to be affected by differences in inherent site characteristics. In the present study, vegetation surveys from the 1950's were compared with recent (2008) surveys in a forest complex in southern Belgium. All plots were situated in ancient forest (defined for Belgium as being continuously forested at least since the oldest available set of topographic maps of 1777; Hermy *et al*., 1999), so we can assume that the herb layer is well developed and adapted to the site. To gain a better insight into the changes of the herb layer on different site types with more or less similar external drivers, we selected plots on two site types in the same forest complex. Because of the proximity of both site types, the changes between the surveys in acidifying and eutrophying deposition, management and grazing density will be similar for both types. First, abiotic, litter and overstorey conditions were compared between the two site types. Next, we investigated herb layer diversity and a set of plant-derived variables (CSR signature, Ellenberg indicator values, etc.) to get a better understanding of the changes of the herb layer. Finally, we compared the shifts of individual plant species on both site types.

2.2 Materials and methods

2.2.1 Study site

The study area was a part of a large forest complex in the south of Belgium (Gaume region, described in chapter 1.3.1).

Between 1945 and 1963, a vegetation survey was done in the south of Belgium to make vegetation maps. We used this vegetation map and classification (Dethioux and Vanden Berghen 1966), and the soil map (Service Public de Wallonie 2007) to select two different forest types in our study area: (1) an acidic site type with acidophilous beech and oak forests (originally called '*Chênaie sessiliflore*' and '*Hêtraie à luzule blanche*'; Dethioux and Vanden Berghen 1966) and (2) a moderately acidic site type with more nutrient-rich conditions supporting moderately acidic beech and oak hornbeam forests (called '*Hêtraie à aspérule*'; Dethioux and Vanden Berghen 1966). For the resurvey in July 2008, we used the vegetation recordings of the cartographical survey located in the selected forest types in the study region. Before the field survey, we selected plots from the old survey with full description of the herb, shrub and tree layer and with a description of the location of the plot. Next, the plots were relocated as well as possible based on the name of the forest part, description of the site, elevation and orientation. Plots were not permanent, which is common in forest resurvey studies (cf. Verheyen *et al.*, 2012), and our study should be regarded as a repetition of the old recordings with equal sampling efforts in the same locations, using the same methodology. On the field, plots with recent management disturbances and plots that were clear-felled in the period between the two surveys were rejected for resurvey. Finally, a total of 43 plots remained for analyses, 16 were located in the acidic site type and 27 in the moderately acidic site type. All the re-inventoried plots in the study area were first surveyed in 1953–1954. The plot size in the old survey was mostly 100 (22 plots out of 43) or 200 m^2 (16 plots), some plots were 50 (1), 300 (2) or 400 m^2 (2). We kept the plot sizes in the recent survey equal to that of the old survey. Analysis without the smallest (50 m^2) and largest (300-400 m^2) plots gave nearly identical results (results not shown) compared to the results presented below.

2.2.2 Field survey and data preparation

Three vegetation layers were recorded, i.e. the herb ($\leq$ 1 m, including brambles, ferns and tree saplings), the shrub (> 1 m and $\leq$ 7 m) and the tree layer (> 7 m). For some analyses, the tree and shrub layer were combined and further referred to as ‘overstorey layer’. In both surveys, the classical Braun-Blanquet scale (7 cover classes) was used to estimate the cover of plant species. For the analyses, the Braun-Blanquet scale was back-transformed to midrange values.

The old survey was made between May and July and the resurvey in July. Some of the spring-flowering forest herbs were not visible in the recent survey. Therefore, we opted to exclude the spring-flowering forest herbs from further analysis in both the old and recent vegetation recordings (i.e. *Adoxa moschatellina, Anemone nemorosa, Anemone ranunculoides, Corydalis solida, Gagea lutea, Neottia nidus-avis, Orchis mascula, Ranunculus ficaria*). We defined the species pool as all species appearing on a site type in a survey period.

To describe the site types, the soil pH and the litter quality were measured in the recent survey. Four soil samples (0–5 cm) were taken on the diagonals at about two meter of each corner of a plot and a mixed sample was analyzed for pH_{KCl} (1 M KCl, 1:5 suspension, ion-specific electrode). The thickness of the litter layer was calculated as the mean thickness of four measurements in the plot. The humus type was determined according to Jabiol *et al*. (1995).

To study changes in the canopy, we calculated the sum of the percentage cover of species in the shrub and tree layer, and two tree functional traits, being the average shade casting ability index and the average litter quality index. The shade casting ability index (SCA) is a species-specific, expert-based index that varies from 1 to 5 (low to high shade casting ability of the canopy species). The litter quality index (LQ) is a similar index also ranging from 1 to 5 (very slow to very fast litter decomposition) (indices scores listed in: Verheyen *et al*. (2011)). For each plot, the mean values of the SCA and LQ indices were calculated based on the index of each individual tree and shrub weighted by its cover (see also Van Calster *et al.*, 2008; Baeten *et al.*, 2009a; Verheyen *et al.*, 2012).

To get a better understanding of underlying causes of changes in the herb layer we calculated plant-derived variables related to the prevailing environmental conditions (Ellenberg indicator values) and dominant plant strategies (CSR-signature). As those variables reflect the environmental conditions of a site, their changes allow to make assumptions about which external drivers underlie the shifts in community composition. Ellenberg indicator values (Ellenberg and Leuschner, 2010) express individual species requirement along seven abiotic gradients. We used Ellenberg indicator values for light (L), soil reaction (R) and nitrogen state of the soil (N, often interpreted as general soil fertility) (Hill *et al.*, 2000; Ellenberg and Leuschner, 2010). Ellenberg average indicator values (AIV) were calculated as weighted (by cover) averages of the indicator values of the herb species occurring in each plot. Weighted (using abundances) and unweighted (using presence/absence data) averages were strongly correlated for each of the three Ellenberg AIV's ($R^2 > 0.8$, $P < 0.001$, n = 43). Furthermore, we calculated the functional signature for each plot based on the CSR-signature and the cover of each plant species following Hunt *et al*. (2004). This approach categorises species into 8 categories based on three primary strategies and their combinations (C-S-R strategies: competitive – stress-tolerant – ruderal) (sensu Grime, 2001).

2.2.3 Statistical analysis

The analyses were composed of three major parts. (i) First, we described the two site types based on abiotic characteristics and the overstorey layer and looked to the differences in overstorey composition, shade casting ability and litter quality between the old and recent survey; (ii) Next, we examined the temporal differences in the herb layer for the two sites with multivariate techniques and compared plant-derived explanatory variables; (iii) Finally, we looked into shifts in individual herb layer species on both site types.

(i) Because of the small sample size and non-normality of some variables, non-parametric Wilcoxon rank-sum tests were used to compare soil and litter characteristics between the acidic and moderately acidic forest types. In a multivariate Detrended Correspondence Analysis (DCA), we visualised differences in overstorey composition. DCA is an eigenvector ordination technique which ordinates simultaneously both species and plots by a re-iterative weighting procedure (Hill, 1979; Hill and Gauch, 1980) and was executed with Canoco (ter Braak and Šmilauer, 2002). A permutation multivariate analysis of variance (PerMANOVA, Anderson, 2001; McArdle and Anderson, 2001) was used to test whether the overstorey composition was significantly different between the two forest types and between the survey periods. We performed PerMANOVA with the *adonis* function in the *vegan* library (Oksanen *et al.*, 2010) of R (R Develompment Core Team, 2010) using Bray-Curtis dissimilarities.

(ii) The herb layer differences between the two survey periods were examined for both site types. First, we characterise the herb layer diversity using two diversity indices: species richness (number of species per plot, α-diversity) and the species pool for a site type and survey period (γ-diversity). Next, we did a multivariate analysis of the herb vegetation data by using again PerMANOVA and DCA. We correlated the Ellenberg indicator values and CSR strategies with the plot-scores of the first two DCA-axes to get an insight in the differences between the two sites and in the shifts of the vegetation between the survey periods. Also the pH values of the recent survey (no data for the old survey) were correlated with the plot-scores of the first two DCA-axes. Finally, the differences in plant-derived variables (Ellenberg indicator values, CSR) between the survey periods were examined for both site types. Wilcoxon rank-sum tests were used to compare the diversity indices and plant-derived variables between the old and recent survey.

(iii) We looked into species-specific shifts in the herb layer. Following Wiegmann and Waller (2006), we used the log likelihood ratio (G-test) goodness of fit test to identify the 'winners' and 'losers' i.e. those species that increased or decreased significantly between the two surveys. Because the small species pool on the acidic sites, this was only done for the moderately acidic sites

For DCA, PerMANOVA and the identification of winners and losers, only species that appeared in at least 15% of the plots in the old or recent survey were used (moderately acidic sites: 65 species; acidic sites: 28 species).

2.3 Results

2.3.1 Abiotic site characteristics and overstorey vegetation

The acidic sites had a significantly lower pH than the moderately acidic sites (pH_{KCl} 3.23 and 4.44 respectively, $P < 0.001$) and a significantly thicker litter layer (30 and 20 mm respectively, $P = 0.006$) (Figure 2.1). The dominant humus types were mull types on the moderately acidic site type (most frequent humus type: oligomull (13/27)), while more moder types occurred on the acidic sites (most frequent humus type: hemimoder (10/16)).

The species composition of the overstorey layer differed marginally significant between the two surveys (PerMANOVA, $P = 0.089$). The most abundant tree species in both surveys were *Fagus sylvatica* (old survey: 38% of the total tree and shrub cover; recent survey: 34%), *Quercus* (*Quercus petraea* and *Quercus robur*) (old survey: 13%; recent survey: 15%), *Carpinus betulus* (old survey: 16%; recent survey: 12%) and *Corylus avellana* (old survey: 13%; recent survey: 13%). In the new survey, *Acer pseudoplatanus* became also became an important tree species (old survey: 2%; recent survey: 10%). The two site types were clearly different in the overstorey layer (PerMANOVA, $P = 0.001$). The DCA of the tree species (Figure 2.2) showed that *Quercus petraea, Sorbus aucuparia, Rhamnus frangula* and *Betula* spp. were more abundant or had higher cover values in the plots on the acidic sites, while *Quercus robur*, *Carpinus betulus, Corylus avellana, Fraxinus excelsior* and *Prunus avium* were more associated with the moderately acidic sites. The litter quality (LQ, Table 2.1) was higher for the plots on the moderately acidic sites compared to the acidic sites.

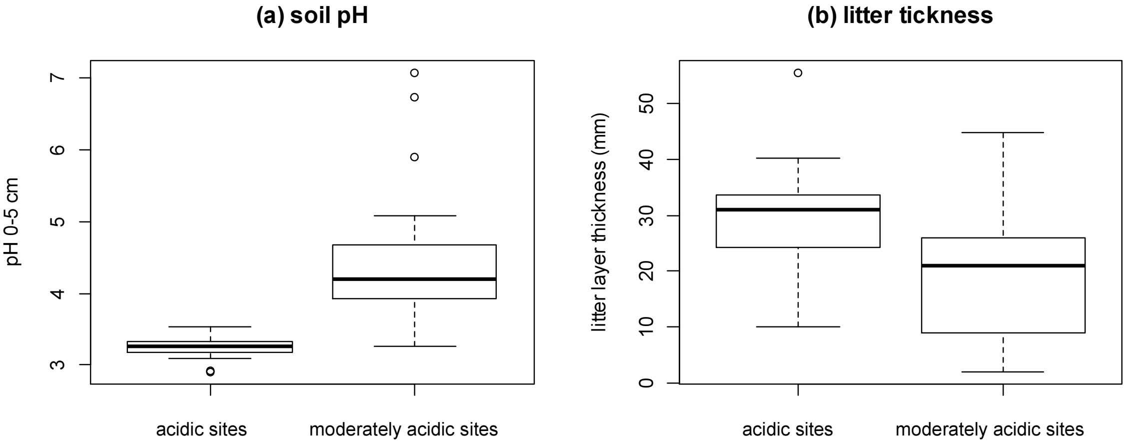


Figure 2.1. Boxplots of a. the soil pH (pH_{KCl}) and b. litter thickness on the moderately acidic and acidic sites.

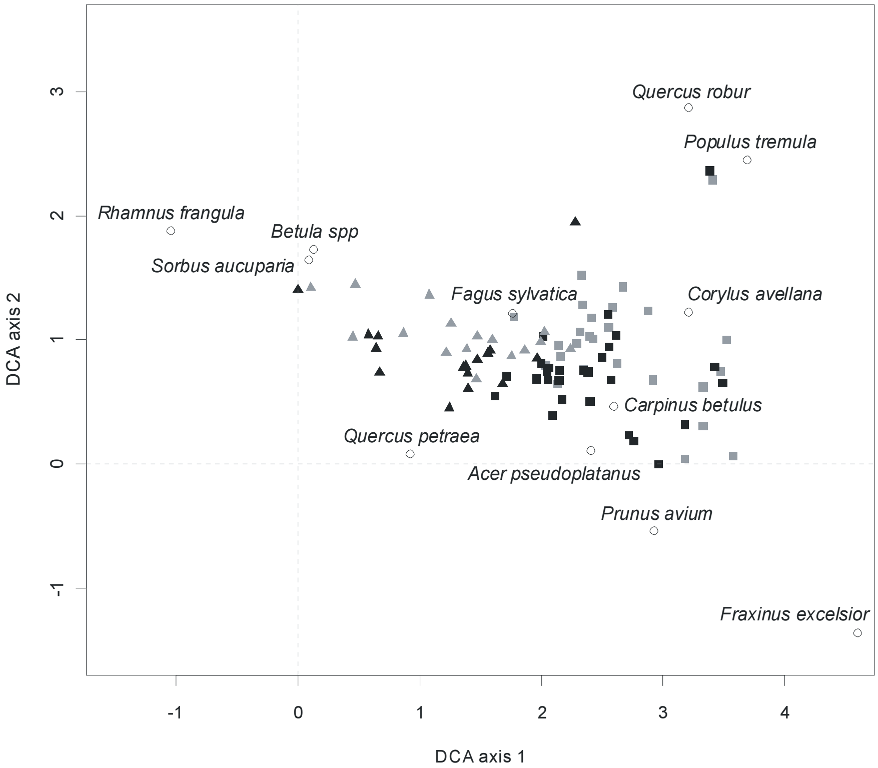


Figure 2.2. Detrended correspondence analysis (DCA) of the tree and shrub layer. Biplot of the plots and the tree species. Species: tree species with mean cover > 2% are plotted. Plots: black symbols are for the old survey and grey symbols for the recent survey period; the triangles are the plots on the acidic sites and the squares are the plots on the moderately acidic sites.

The cover of the overstorey layer stayed comparable, but the cover of the tree layer increased significantly on the moderately acidic sites. The cover of the shrub layer showed a small decrease on both site types. We could only find temporal changes in LQ and SCA for the tree layer of the moderately acidic site type, the shade casting ability became lower and the litter quality became better (Table 2.1). There was no interaction between site type and the survey time (PerMANOVA, $P = 0.499$).

2.3.2 Understorey vegetation shifts

For both site types, the species pools of the old survey were larger than those of the recent survey. The mean species diversity per plot in the old survey was significantly higher than the recent survey for the moderately acidic site type, while there was no significant difference for plots on the acidic site type (Table 2.1).

The two site types could be clearly differentiated along the first DCA-axis (length of gradient: 4.5 S.D.; explaining 15% of the variation in species data, Figure 2.3). This first axis was strongly correlated with AIV_R ($\rho = 0.84$; $P < 0.001$), AIV_N ($\rho = 0.69$; $P < 0.001$), CSR_R ($\rho = 0.85$; $P < 0.001$) and pH (only pH-values available for the recent survey: $\rho = 0.88$; $P < 0.001$). This first axis indicated a gradient of soil acidity and nutrient availability with the acidic sites on the side with low pH and lower nutrient levels, and the moderately acidic sites on the side with higher pH and nutrient levels. The old and recent surveys were clearly distinguishable along the second DCA-axis (explaining 6% of the variation in species data), but the correlations of the variables with this axis where smaller. CSR_C ($\rho = -0.43$; $P < 0.001$), AIV_N ($\rho = 0.34$; $P < 0.001$), CSR_S ($\rho = 0.34$; $P = 0.001$), AIV_L ($\rho = 0.31$; $P = 0.004$) and AIV_R ($\rho = 0.30$; $P = 0.004$) were correlated with this second axis. This means that the understorey vegetation shifted towards more competitive strategy and less stress-tolerant strategy, and towards lower light levels and higher pH and nutrient levels in the plots. Also the PerMANOVA-analysis showed a significant difference between the site types (Pseudo-$F = 16.08$; 6.53; $P \leq 0.001$) and between the old and the recent survey (Pseudo-$F = 6.53$; $P \leq 0.001$). The interaction between survey time and site type was also significant (Pseudo-$F = 2.45$; $P = 0.007$), indicating that the two site types differentially changed over time. From the DCA (Figure 2.3), it can be deduced that the differences between the old and recent survey were larger for the moderately acidic forest types than for the acidic sites.

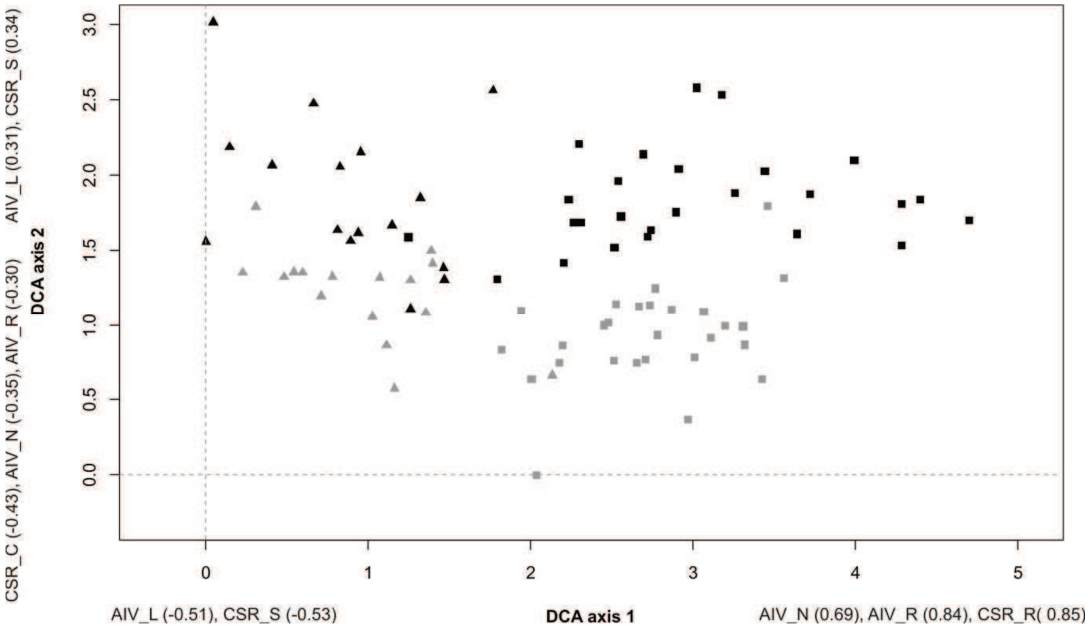


Figure 2.3. Scatterplot of DCA of the herb layer. Plot scores on the first and second DCA-axis of old (black) and new (grey) surveys. Triangles are plots on the acidic sites type, squares are plots on the moderately acidic sites type. Explanatory variables correlated significantly (*P* < 0.05) with the DCA-axis are listed on the axis with their spearman's rank correlation coefficient. Average Ellenberg indicator values for light (AIV_L), soil reaction (AIV_R) and nitrogen state of the soil (AIV_N). CSR-strategy: value for stress-tolerance (CSR_S), competive signature (CSR_C) and ruderal signature (CSR_R).

Comparing Ellenberg indicator values between the old and recent survey showed only significant differences for the moderately acidic forest types. The trend of the herb layer changes on the acidic site types was equal, but not large enough to be significant. For the moderately acidic forest sites, the AIV_L significantly decreased and AIV_N and AIV_R increased in the recent survey (Table 2.1). This can be interpreted as a shift of the understorey layer on the moderately acidic sites to plants requiring less light, more nutrients and more basic soil conditions. The changes in CSR-signature between the old and new survey were similar for both site types. There was a significant increase of the 'competitive' (C)-strategy and a decrease of the 'stress-tolerant' (S)-strategy and the 'ruderal' (R)-strategy.

2.3.3 Species-specific changes

Most significant changes in the herb layer species were found on the moderately acidic sites. In those plots, 16 losers and only 4 winners were found (Figure 2.4). The herb layer species that increased significantly in cover and abundance are *Corylus avellana*, *Circaea lutetiana*, *Galeopsis tetrahit* and *Stellaria nemorum*. On the side of the losers, 9 species which occur at least six times in the old survey were absent in the recent survey. The small number of winners compared with the larger group of losers did not allow us to make a statement about characteristics of these groups. But, it is clear that the species that were abundant in the old survey and decreased or disappeared in the recent survey, are species that occur in forests in the more open sites (clear-felled areas and forest edges) or in forests with high light availability on the forest floor (e.g. *Scrophularia nodosa, Poa chaixii, Euphorbia amygdaloides* and *Veronica chamaedrys*).

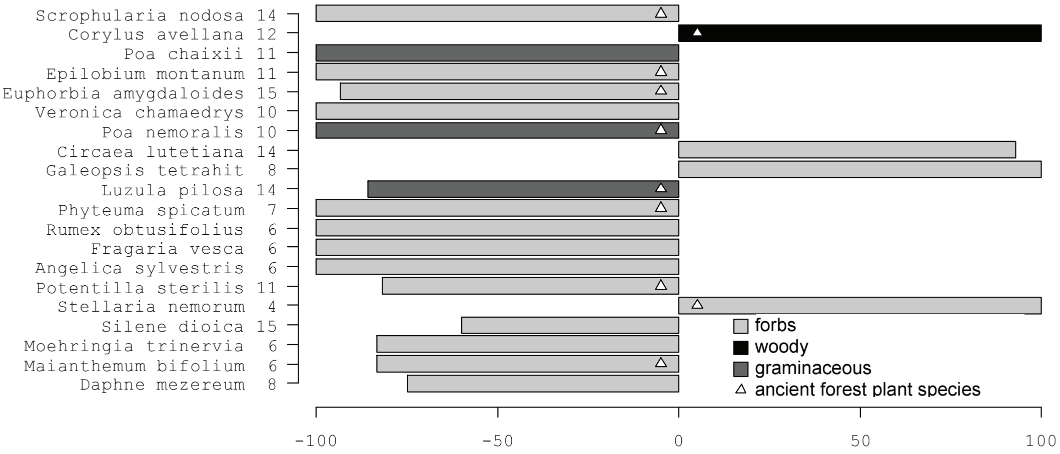


Figure 2.4. Changes in the frequency of winner and loser species of the moderately acidic sites. Species ordered from highly significant to less significant changes in frequency. Numbers shown on the y-axis-labels represent the highest frequency in the old or recent survey (winners: frequency in recent survey, losers: frequency in old survey). Ancient forest plant species according to Hermy *et al*. (2009).

Table 2.1. Changes in diversity and plant-derived variables of the herb layer and overstorey variables for the acidic and moderately acidic site types between the old and recent vegetation survey (Wilcoxon rank-sum test). Significance: NS, not significant; (*) P ≤ 0.10; * P ≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001. Overstorey variables: LQ, litter quality index; SCA, shade casting ability index.

	Acidic site type				**Moderately acidic site type**			
	mean		Statistic	significance	mean		statistic	significance
Survey	old	recent			old	recent		
				Species diversity and cover				
Species pool	64	46			113	99		
Species richness	13.6	12.3	147	NS	26.2	20.5	565	***
				Average Ellenberg indicator values (AIV)				
Light (AIV_L)	5.31	5.11	138	NS	4.04	3.70	479	*
Nitrogen (AIV_N)	3.32	3.42	153	NS	5.01	5.51	163	***
Soil reaction (AIV_R)	2.62	2.93	98	NS	5.61	6.16	210	**
				CSR-strategy				
Competitive signature (C)	0.40	0.58	48	**	0.39	0.49	105	***
Stress-tolerance (S)	0.56	0.40	203	**	0.43	0.33	551	**
Ruderal signature (R)	0.04	0.02	186	*	0.17	0.18	361	NS
				Overstorey layer				
Cover overstorey	102.28	105.42	127	NS	128.65	134.87	341	NS
Cover trees	69.46	86.78	98	NS	74.67	96.23	226	*
Cover shrubs	32.81	18.64	177	(*)	56.06	38.64	433	NS
SCA overstorey	3.67	3.72	136	NS	4.13	3.97	440	NS
SCA trees	3.85	3.64	144	NS	4.18	4.09	462	(*)
SCA shrubs	3.96	4.16	98	NS	3.86	3.66	415	NS
LQ overstorey	1.67	1.65	135	NS	2.27	2.34	305	NS
LQ trees	1.45	1.60	98	NS	1.91	2.28	189	**
LQ shrubs	2.03	1.79	153	NS	2.88	2.75	444	NS

2.4 Discussion

The two site types were clearly different with regards to soil and litter characteristics as well as over- and understorey community composition. The higher pH, better litter quality and thinner litter layer on the moderately acidic sites are indicators of a more nutrient-rich system. The differences between the site types are probably explained by the variation in depth and lime content of the substrate and different soil texture classes. The overstorey composition will be influenced by this geomorphic variation (Ellenberg and Leuschner, 2010) and by management, but on the other hand, composition differences of the overstorey layer will have an effect on the soil pH and the litter layer (Reich *et al.*, 2005). Both geomorphic differences and overstorey composition are interacting and resulted into two site types with related forest types. The temporal differences of the overstorey layer were not significant. The small differences in cover of the tree species could occur due to observer bias, the use of semi-permanent plots or due to forest management or succession. Despite that the plots are not permanent, the species composition of the tree layer was similar enough to have no effects on the herb layer. In contrast with the unchanged overstorey species composition, there were significant changes in the herb layer diversity and composition, especially on the moderately acidic site type.

In forest in western and central Europe, the species richness generally increases with higher pH (Brunet *et al.*, 1997; Ewald, 2003; Schuster and Diekmann, 2003; Partel *et al.*, 2004; Kooijman, 2010). In this study, the most diverse plots were also located on the moderately acidic sites. The local species pool declined for both site types over time. However, the average species diversity in a plot decreased significantly only at the moderately acidic sites, and there were significantly more losers than winners. This result is consistent with other studies performed in Belgium and northern France that found decreasing species diversity over time (e.g. Lameire *et al.*, 2000; Van Calster *et al.*, 2008; Baeten *et al.*, 2009a; Baeten *et al.*, 2010a), although Thimonier *et al.* (1994) found an increase of diversity. Moreover, a recent meta-analysis of herb-layer resurvey studies in deciduous forests revealed that there was no clear directional temporal change in the understorey diversity across Europe (Verheyen *et al.*, 2012). In the present study, we showed that changes of species diversity between site types can differ markedly, even within a small region.

The direction of the changes in plant-derived variables was similar on both sites, but changed most on the moderately acidic sites: the community composition shifted towards more N-demanding and shade-tolerant species. This trend is similar to the overall tendency of understorey vegetation shifts in deciduous forests in western-Europe (Verheyen *et al.*, 2012). Contrary to our expectations, the AIV_R increased, suggesting an increasing pH. However, we believe that this increased value does not necessarily mean that the soil pH has effectively increased. This could be explained by following three causes: First, AIV_R and AIV_N were strongly correlated (R^2 0.84, $p<0.001$, $n = 86$). Thimonier *et al.* (1992) suggested that, even if there was a small decrease or no change of pH, we probably would not be able to detect this change in the AIV_R because the correlation with the strongly increased AIV_N. Secondly, as mentioned by Diekmann (2003), an acidification of the vegetation is not always indicated by a decreasing AIV_R, because eutrophication and lower management intensity may lead to denser canopies which causes the disappearance of light-demanding species which often have lower R-values. Thirdly, some temporal vegetation studies with a decreasing soil pH (based on pH measurements for both survey periods) also showed an increased or unchanged AIV_R value (Van Calster *et al.*, 2007; Baeten *et al.*, 2009a).

Below, we first look to the key drivers of these shifts, and then we discuss the potential causes of the different shifts between the two site types. In most vegetation studies in ancient forests in Western-Europe, temporal shifts in understorey vegetation could be attributed to one or more of the three main key drivers: (i) the input of potentially acidifying and eutrophying deposition and (ii) changes in management type and intensity or (iii) changes in grazing densities. The last one seems not applicable for this study area. Significant changes in browsing and grazing intensity by increasing or decreasing deer population (e.g. Kirby and Thomas, 2000) or total closure for deer (Rooney, 2009) have to take place to be able to detect an impact on the understorey vegetation. Based on counts of wild boar and deer by hunters and forest managers, the grazing density was relatively stable over the last 50 years in our study area. Roe deer and red deer densities were estimated to be 16 per 100 ha for the recent survey. So, this key driver could be excluded for this study area. Below, we discuss the two remaining key drivers. Since both factors occur together and interact it is difficult to clearly distinguish the relative importance of the two drivers (Van Calster *et al.*, 2007; Baeten *et al.*, 2009a).

(i) The high input of potentially acidifying and eutrophying deposition during the last decades has caused soil acidification and eutrophication in many temperate forests and these altered soil conditions have changed the herb layer towards a more acidic and eutrophic type (Falkengren-Grerup, 1986; Thimonier *et al.*, 1994; reviewed in: Bobbink *et al.*, 1998; Gilliam, 2006). Also in this study, an increasing AIV_N indicate this shift on the moderately acidic sites. Very likely, deposition is also the cause for the increase of the competitive signature and decrease in stress-tolerance of plots on both site types (Bobbink *et al.*, 1998).

(ii) Forest management became less intensive in many western European forests (e.g. Van Calster *et al.*, 2007; Baeten *et al.*, 2009a; Hedl *et al.*, 2010). Also in our study area, the former coppice or coppice with standards silvicultural system was abandoned in the first decades after the first survey and changed to mixed and uneven aged high forest management, resulting in a lower cover of shrubs and increase of tree cover. Changes in cover and composition of the overstorey layer influence the herb layer mainly by changes in litter quality and increased shading (Van Calster *et al.*, 2007; Baeten *et al.*, 2009a). Also for this study, these changes very likely caused shifts in the herb-layer vegetation towards more shade-tolerant plant species and may, to some extent, also explain the eutrophication signal (Verheyen *et al.*, 2012).

Although shifts in understorey vegetation differed between the moderately acidic and acidic site type, the input of potentially acidifying and eutrophying deposition and the management change did not. Therefore, these two key drivers are unable to explain the differences in shifts between the two site types. We argue that the differences between the two site types mainly occur due to variation in site type and related differences in abiotic and biotic conditions. We suppose that the external drivers of change could have a different impact depending on the soil type and herb vegetation. Below, we discuss the origin of the differences between the site types in detail.

Input of acidifying deposition may lead to losses of buffer capacity and lower pH, but soils have an inherent potential to buffer acidity depending on e.g. soil type and parent material (Ulrich, 1983b). The acidic sites were situated mostly in the hydroxy-aluminium buffer range, while the moderately acidic sites were situated in the cation exchange buffer range at the time of the last surveys. A similar accumulated acid load has been shown to cause different pH-changes in different buffer ranges (Bowman *et al.*, 2008). Variation in soil characteristics, e.g. texture, pH and nutrient status, interact to determine the sensitivity to a higher N-input (Matson *et al.*, 2002; Bobbink *et al.*, 2010). The higher sensitivity for acidifying input of the better buffered soils is also shown in the next chapter of this thesis. In addition, there are also differences in sensitivity of plants to acidification and eutrophication. Plant communities often change due to N-input, probably through various ecological mechanisms (Matson *et al.*, 2002). Increased N availability favours the growth and dominance of fast-growing, nutrient-rich species over slow-growing nutrient poor ones, with resulted in a decline of the species richness

(Tilman, 1997, Aerts and Berendse, 1988). Hence, a first cause for the different shift at the two site types was that the soil and vegetation of these two site types showed a different response to an equal N-input.

Furthermore, the effect of changes in management type and intensity could differ between site types. Generally, lower management intensity allowed the forest to shift to more developed forests. It is known that the peak in plant diversity during succession may vary with site quality (Roberts and Gilliam, 1995). In the specific case of conversion from coppice to high forest, Van Calster *et al.* (2007; 2008) compared different conversion types on two site types. They observed different shifts of the soil characteristics and the herb layer between conversion to even and uneven aged high forest and for some variables also between the site types. Changes in tree species cover and composition had a significant influence on the direction of these changes. Tree species with base-rich litter could enrich soil and vegetation while species with lower litter quality could induce soil acidification (Reich *et al.*, 2005). But, in our study, the tree species composition, litter quality and shade casting ability of the overstorey did not change significantly. Still, the increased cover of trees on the moderately acidic site could create more shaded conditions for the herb layer and could increase the litter input. The coppice forests were characterised by short cyclic rotations with different levels of light and temperature for different stadia (Ash and Barkham, 1976). Several plant species need these periods with optimal environmental conditions (for example: temporal increase of light after logging) to survive the intermediate, less favourable periods (Salisbury, 1916; Barkham, 1992). Due to a higher productivity on the moderately acidic sites, the management cycles were shorter on these sites, and probably more species were adapted to these cyclic conditions. This could contribute to a higher loss of species and a more significant shift in composition on the moderately acidic sites. The management changes could possibly also be interpreted in the context of the intermediate disturbance hypothesis (Connell, 1978), which proposes that the maximum diversity is maintained at intermediate disturbance size, frequency and intensity. At low disturbance levels, a few competitive species will dominate the ecosystem, whereas only a few species can survive the more extreme conditions at high disturbance levels. In addition, higher levels of diversity are expected when the present disturbance regime resembles the historic regime (Denslow, 1980). Centuries of coppice management resulted in a specific understorey flora in these forests (e.g. Rackham, 1980; Evans and Barkham, 1992). On the moderately acidic sites, the disturbances intensity was high and the frequency intermediate (intermediate situation). Disturbance frequency and especially disturbance intensity will decrease on these sites after conversion, with lower understorey diversity and increased competition as result. On the acidic sites, competitive species increased as well, but α-diversity did not. On this site type, the rotation time in the coppice system was likely longer due to the lower productivity. This contributes to smaller differences between the historic and present management regime, which could explain the smaller effect on the diversity.

Moreover, both site types had a different understorey species pool. Species have specific environmental requirements with different sensitivities (Ellenberg and Leuschner, 2010) or strategies to survive (Grime, 2001). Therefore, also vegetation types with different species could have different sensitivities for environmental changes (Diekmann, 2003) or strategies (Hunt *et al.*, 2004) which made the vegetation to show a different shift on different site types with a similar external change. In the context of conversion from coppice to high forest, Van Calster *et al.* (2008) mentioned also that the initially larger species pool on the rich soils was more sensitive to a decline of demanding species and an increase in acidic or indifferent species. Higher nitrogen input also has variable effects on plants (Gilliam, 2006) which made that different plants, and probably also plant communities, could show different responses to increased nitrogen availability.

Earthworm sampling, Gaume, 2009

3

The biogeochemical effects of deciduous to coniferous forest conversion differ along a gradient of soil buffering capacity

After: Verstraeten G, De Schrijver, A, Hlava J, Wuyts K, Schelfhout S, Verheyen K, Muys B. The biogeochemical effects of deciduous to coniferous forest conversion differ along a gradient of soil buffering capacity. Soil Biology Biochemistry, *submitted.*

Abstract

We assessed how the conversion of mixed deciduous forests to Norway spruce (*Picea abies*) plantations affects soil characteristics, the forest floor mass and the earthworm community. We also investigated whether the effects of this conversion depend on the initial soil buffering capacity. The study area contained ancient deciduous woodlands mainly consisting of *Quercus* spp., *Fagus sylvatica* and *Carpinus betulus* in the Gaume, an area with a mild temperate climate in southern Belgium. Spruce plantations were established 35–50 years ago in this matrix of deciduous woodlands. The forest floor, soil characteristics and earthworm community were compared in paired plots in spruce plantations and adjacent deciduous forest along a soil buffering capacity gradient. The soil clay content varied between 3 and 34 % and was used as a proxy for the natural gradient in soil buffering capacity independent of the vegetation. In the deciduous plots, clay content was correlated with pH, forest floor mass, overstorey layer and earthworm biomass. The topsoil pH was significantly lower in the spruce stands compared to the adjacent deciduous stands. In spruce stands, the exchangeable aluminium and calcium concentrations were higher and lower, respectively. The forest floor mass was between two to ten times higher under spruce. The total earthworm biomass was significantly lower in the spruce stands and endogeic earthworms were mostly absent. Differences between deciduous and spruce stands in soil variables and earthworm biomass were more pronounced on sites with higher clay content, so the initially well buffered soils.

3.1 Introduction

It is known for a long time that vegetation, soil characteristics and soil organisms closely interact and can be viewed as co-developing components of terrestrial ecosystems (Zinke, 1962; Van Breemen, 1993; Reich *et al.*, 2005). In forests, tree species composition influences abiotic and biotic soil characteristics in different ways, among others through leaf litter quality and quantity (Augusto *et al.*, 2002). The chemical composition of leaf litter is an important determinant of the litter decomposition rate: litter rich in calcium and with a low lignin content and C:N ratio decomposes faster (Hobbie *et al.*, 2006). High leaf litter calcium concentrations are also associated with increased earthworm abundance (Reich *et al.*, 2005; De Schrijver *et al.*, 2012). Bioturbation by earthworms is important for the rate of litter decomposition and sustaining mull humus quality (Muys and Lust, 1992), but species with bioturbation activity are sensitive to acidification (Muys and Lust, 1992; Muys and Granval, 1997). Slowly decomposing litter favours the build-up of the forest floor, which results in the production of organic acids as intermediate decomposition products (de Vries and Breeuwsma, 1985) and causes a delay in the cycling of buffering cations (Nilsson *et al.*, 1982). Organic acids leach through the soil profile, where they might cause strong modifications in soil chemical, physical and biological characteristics. Next to litter quality, tree species' specific dry deposition and canopy exchange can cause differences in potentially acidifying input, with generally a higher potentially acidifying input on the forest floor and soil under evergreen coniferous species compared to deciduous species (De Schrijver *et al.*, 2007). Also the amounts of acid exudates by tree roots vary between species. For example, the rhizosphere of Norway spruce (*Picea abies*, further called spruce) is more acid than several other European species like oak and beech (Calvaruso *et al.*, 2011). Tree species composition creates also variation in microclimate by differences in rainfall interception and light irradiance (Porte *et al.*, 2004). So, tree species composition affects several important ecosystem characteristics and processes and therefore, the impact of conversion to another tree species can cascade through the whole forest ecosystem.

The tree species composition of many forests in western and central Europe has been changed since the beginning of the 20th century. Large areas of mixed deciduous forests were converted into conifer plantations offering faster growth of highly demanded wood products (Klimo *et al.*, 2000; Spiecker *et al.*, 2004). In our study area, ancient deciduous forests have also been converted to spruce monocultures. The impact of spruce on forest ecosystem functioning is well-studied. Spruce is generally considered to be a tree species with strong negative ecological effects due to its recalcitrant leaf litter, continuous leaf cover and high leaf area index (LAI) (Ranger and Nys, 1994; Augusto *et al.*, 2002). As a result of its recalcitrant litter, spruce litter decomposes slowly and causes a significant build-up of the forest floor (Gerboth, 1998; Vesterdal *et al.*, 2008); so soil acidification can be expected (Binkley and Valentine, 1991). Soil acidification is related to decreased exchangeable base cations concentrations and increased potentially toxic aluminium concentrations, both detrimental for earthworm communities (e.g. Muys, 1989; van Gestel and Hoogerwerf, 2001; Reich *et al.*, 2005). The high LAI and continuous leaf cover of spruce cause dryer soils and high interception rates of potentially acidifying pollutants (Augusto *et al.*, 2002) that adversely affect the earthworm populations (e.g. Nordstrøm and Rundgren, 1974; Grossi and Brun, 1997; Wever *et al.*, 2001; Eggleton *et al.*, 2009).

Based on this substantial knowledge base from the literature we could expect that the conversion of deciduous forest into spruce plantations causes severe changes in the ecosystem, more particularly soil acidification, an accumulation of the forest floor mass, and a decrease of the earthworm activity, but the question remains how this process is triggered and if this mechanism is the same along a gradient of site conditions. Ulrich and Sumner (1991) pointed already to the fact that soil acidification is not linearly correlated with the acidifying input, but depends on soil buffering capacity. The latter depends on discrete buffer ranges (overview of buffer ranges in Box 1). In sum, the buffering capacity and rate of the soil depends mainly on the availability of $CaCO_3$, the weathering capacity and the proportion of clay and humus that is present in a particular soil. Hence, we could expect a different acidification rate due to spruce trees along a gradient of soil buffering capacity determined by e.g. $CaCO_3$, soil depth and clay content.

We designed an original sampling scheme of paired plots along a natural soil buffering gradient, where each pair consist of a deciduous forest plot and an adjacent plot converted to spruce in recent decades. With such an approach we were able to examine the change trajectories from deciduous to spruce for the soil acidity, the forest floor and the earthworm communities and to study the effects of the soil buffering gradient on them.

3.2 Materials and methods

3.2.1 Study site

The study area was a part of a large forest complex in the south of Belgium (Gaume region, described in chapter 1.3.1).

3.2.2 Study design and data collection

To evaluate how forest conversion from deciduous forest to spruce plantation affected the forest floor and variables related to soil acidification, we compared unconverted (deciduous) and adjacent converted (spruce) stands in fourty pairs along a gradient in soil buffering capacity. The plots were selected in a two-step approach: First, we identified all the spruce stands in the study region on aerial photographs and topographical maps; stands at the outer edges of the forest complex were excluded to avoid edge effects. As such, only tree stage stands of 35-50 years old, so comparable with the deciduous stands, were retained. The soil texture map of the Service Public de Wallonie (2007) and the vegetation map of Dethioux and Vanden Berghen (1966) were used to select the plots along a broad range in soil buffering capacity.

In a second step, we examined all preselected spruce and adjacent deciduous stands in the field. Paired deciduous and spruce stands ought to have similar elevation, slope and slope orientation. Deciduous stands that were clearcut after the start of conversion (50 years ago) were omitted. An important assumption in this study design was that, just before planting the spruce trees, both stands were comparable in forest history, tree species composition, vegetation and abiotic characteristics. This was verified *ex ante* by comparison of soil and vegetation maps and forest management archives, by *in situ* comparison of soil profiles and by *ex post* comparison of the chemical soil properties of the deepest soil layer sampled (25-35 cm; see below), and resulted in 40 deciduous-spruce pairs.

In each of the selected paired stands, a squared 10 m x 10 m plot was set out, of which the centre was always 30 m (about one tree height) from the border into both the deciduous and coniferous stands to minimize plot differences as well as mutual influences between the stands. In June 2009, the cover of all species in the herb, shrub and tree layer was recorded in the squared plots, but only the tree layer data are used in this study. Soil variables were determined by analysing soil characteristics at three depth intervals (0 – 5 cm, 10 – 20 cm and 25 – 35 cm). The plots were divided in four parts by the diagonals and for each depth interval, one sample was randomly taken in each part pooled into a composite soil sample. The soil samples were dried at 40°C until constant weight and analysed for pH (extraction in 1 M KCl, 1:5 suspension, ion-specific electrode) at the three depths and exchangeable concentrations of Ca^{2+} and Al^{3+} (extraction in 0.1 M $BaCl_2$, 1:20 suspension, Dutch standard method NEN 5738; flame atomic absorption spectrophotometry, SpectrAA-220, Varian) for the 0 – 5 cm and 25 – 35 cm layer. The soil texture of the 10 – 20 cm depth interval (expressed in percentages clay, sand and loam) was determined by laser granulometry (Malvern Mastersizer S). The litter (L), fragmentation (F) and humus (H) layer of the forest floor were collected separately in four squares of 20 cm x 20 cm in each plot. These samples were dried at 70°C until constant weight and weighted. The total forest floor mass was the sum of the three layers.

The earthworm community was determined in thirty of the forty deciduous-spruce pairs. Earthworms were sampled at two opposite corners of the vegetation plot. The sampling was performed in three steps: first a 0.5 m² square was put on the ground in which the vegetation was carefully removed. From a smaller square of 0.1 m² positioned in the middle of the big square, the forest floor was collected and stored in plastic bags; the forest floor outside that small square was removed. The earthworms in the collected forest floor were retrieved by hand-sorting. Next, we poured 60 litre of mustard solution per plot (6 g mustard powder L^{-1}) in the big 0.5 m² square (Valckx *et al.*, 2011). The frequency of pouring depended on the percolation rate of the solution. The earthworms were collected directly after emerging. Finally, a soil monolith (0.1 m² and 20 cm deep) in the mustard affected area was thoroughly checked for earthworms by hand-sorting. All the earthworms were first stored in 95 % ethanol and after a few hours transferred to a 5 % formalin solution for fixation. After 3 days they were transferred to 95 % ethanol for further preservation and identification. In the laboratory, the earthworms were identified to species level following the nomenclature of Sims and Gerard (1999) and they were weighed with gut contents. Earthworm mass was expressed as g m^{-2} formalin-preserved weight. In the few cases that identification to species level was not possible, the earthworms were identified at genus or ecological group level and were assigned pro rata to species level (cf. Valckx *et al.*, 2006).

3.2.3 Data analysis

To compare the means of the different variables we used non-parametric Wilcoxon tests to test whether the means of two sets of paired measurements are significantly different. Correlations were calculated by Pearson's correlation in case of normality and the presence of a linear relationship and Spearman's rank correlation in other cases. We extracted the first axis of a Detrended Correspondence Analysis (DCA) on the overstorey vegetation in the deciduous stands (canopy cover data) as a proxy for the overstorey composition. DCA is an eigenvector ordination technique which ordinates simultaneously both species and plots by a re-iterative weighting procedure (Hill, 1979).

We produced a synthesis diagram where we looked at the relations between several variables of both deciduous and spruce stands together. We calculated standardised regression coefficients to visualize relations between tree species, forest floor, soil and earthworms. Standardised regression coefficients express the standard deviation change in a response variable associated with the change in standard deviation change of a predictor variable and this makes the relations between the variables more comparable. For this diagram, we created a new variable (*tree composition* * *clay*) that takes both the acidifying input of the tree layer as well as the acidification potential of the soil into account. Thus, we combined the clay content of the soil (as a proxy for the acidification potential) with the first DCA axis of the percentages tree species cover in a plot. Plots with high cover of acidifying species (spruce) had low values on the first DCA-axis and plots with the highest percentage cover by the least acidifying species (e.g. *Fraxinus excelsior*, *Prunus avium*) had high values on the first DCA-axis. Plots with low clay content and acidifying trees had low values for this variable, plots with high clay content and least acidifying species had high values. All statistical analyses were performed with R 2.15.2 (R Development Core Team 2010).

3.3 Results

3.3.1 Site gradient

The clay content in the soil varied between 3 and 34 % and was used as a proxy for the natural gradient in soil buffering capacity independent of the vegetation. This gradient was correlated with soil variables concerning acidification, the forest floor, the overstorey composition and the earthworm community in the deciduous stands (Table 3.1). The clay content of the soil was significantly and positively correlated with the mineral topsoil (0 –

5 cm) pH-KCl. Both variables showed similar correlations with the soil acidification parameters, the forest floor and the earthworm biomass, but correlations were the strongest for pH-KCl. The pH-KCl in the deciduous stand varies between 3.2 and 4.7 (Figure 3.1). The pH-KCl and the clay content were significantly correlated with the calcium concentration of the mineral topsoil (0 – 5 cm) in the deciduous stands, but not with the aluminium concentration. The relation between pH and the aluminium concentration was a unimodal relationship with a maximum concentration around pH-KCl 3.6. Also the overstorey composition (first axis of the DCA of the deciduous overstorey layer) was significantly correlated with the clay content and the mineral topsoil pH-KCl in the deciduous stands. The species composition varied from stands dominated by *Q. robur* in plots with low clay content to a species mixture with species as *F. excelsior* and *P. avium* in plots with higher clay content. The total forest floor mass and the biomass of the fragmentation and the humus layer in the deciduous stands decreased with increasing clay content and pH-KCl of the soil (Table 3.1). The litter layer mass of the forest floor was not correlated with the clay content and pH-KCl. There was no H-layer in 18 out of the 40 deciduous plots. Both the endogeic en epigeic earthworm biomasses in the deciduous stands were positively correlated with the topsoil pH-KCl and the clay content (Table 3.1).

Table 3.1. Correlations (Spearman rho correlation coefficient) between clay content (at 10 – 20 cm), variables related to soil acidity in the topsoil (0 – 5 cm), the overstorey composition, the forest floor and the earthworm community in the deciduous stands. FFM: forest floor mass. Topsoil: 0 - 5 cm layer. Significance: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

		Clay content of the soil		**Topsoil pH**	
Topsoil	pH-KCl	0.65	***		
	Log (topsoil calcium concentration)	0.58	***	0.84	***
	Topsoil aluminum concentration		NS		NS
Overstorey vegetation	First DCA axis overstorey	0.56	**	0.87	***
Forest floor	Total forest floor mass	- 0.55	**	- 0.79	***
	FFM - litter layer		NS		NS
	FFM - fragmentation layer	-0.54	**	-0.71	***
	FFM – humus layer	-0.52	**	-0.72	***
Earthworm community	Endogeic earthworm biomass	0.54	**	0.84	***
	Epigeic eartworm biomass	0.49	**	0.59	***

3.3.2 Conversion effects

The soil pH-KCl in the spruce stands was significantly lower than in the deciduous stands for both the 0 – 5 cm and the 10 – 20 cm soil layers (Figure 3.1). The exchangeable aluminium concentrations were significantly higher and the exchangeable calcium concentrations were significantly lower in the topsoil (0 – 5 cm) in spruce stands. In the deepest measured soil layer (25 - 35 cm), the soil pH-KCl and calcium concentrations did not differ significantly (Figure 3.1) while the aluminium concentration was significantly higher under spruce, but the within-pair difference was smaller than in the topsoil.

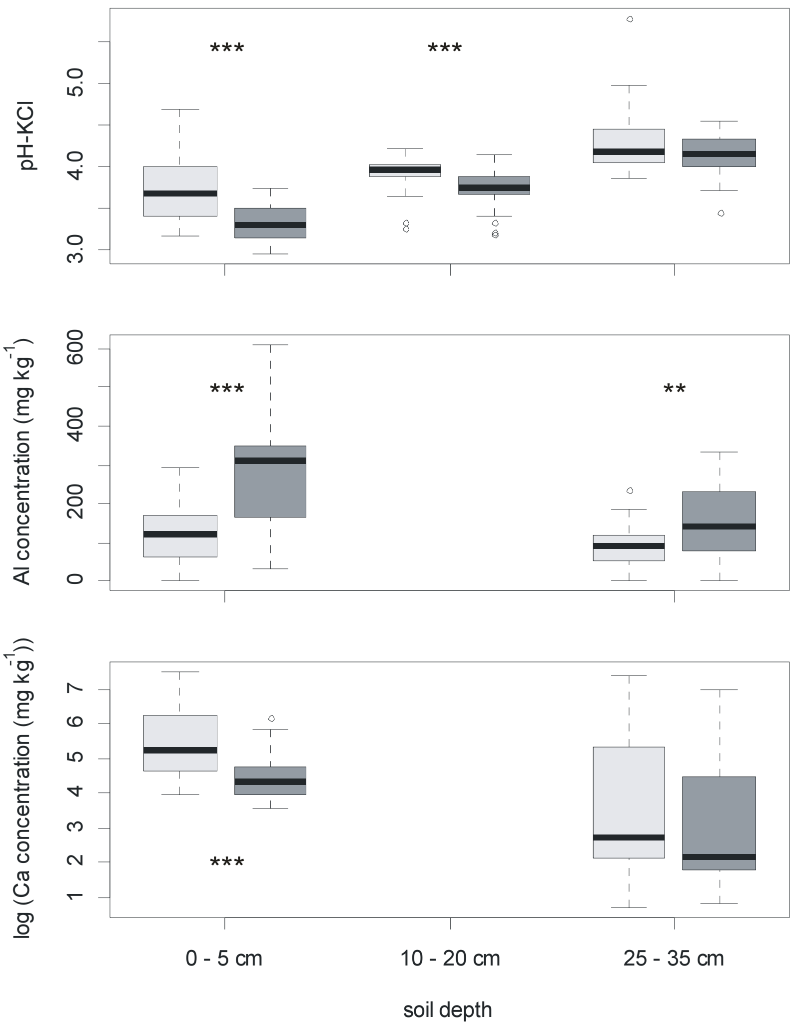


Figure 3.1. Comparison of pH, calcium and aluminium concentrations in different soil layers of deciduous (light grey) and spruce (dark grey) stands (number of pairs = 40). Significant differences are shown (Wilcoxon signed-rank test): * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

The forest floor mass was between two to ten times higher in the spruce stands compared to the deciduous stands. The differences in biomass were also significant for the L, F and H-layers separate, although most obvious and significant for the H-layer (Figure 3.2).

The total earthworm biomass was significantly lower in the spruce stands compared with the adjacent deciduous stands. Anecic earthworms were only found in one deciduous forest plot. Endogeic earthworm species were almost absent in plots with topsoil pH-KCl values lower than 3.5 and their mass strongly increased in soils with pH-KCl higher than 4. The endogeic earthworms were mostly absent in the spruce stands where also their biomass was significantly lower compared to the deciduous stands. Epigeic species were present in both coniferous and deciduous forest stands, but their biomass was significantly lower in the spruce stands, mostly between 1.5 to 5 times lower under spruce (Figure 3.3).

3.3.3 Interactions between site and conversion effects

The differences between deciduous and spruce stands in soil pH, exchangeable aluminium and calcium concentrations were more pronounced on sites with higher clay content (Figure 3.4). Plots with the lowest clay content exhibited only small differences within a deciduous-spruce pair: about less than 0.2 pH unit. At the other side of the clay gradient, differences were about 0.8 pH units within a deciduous - spruce pair (Figure

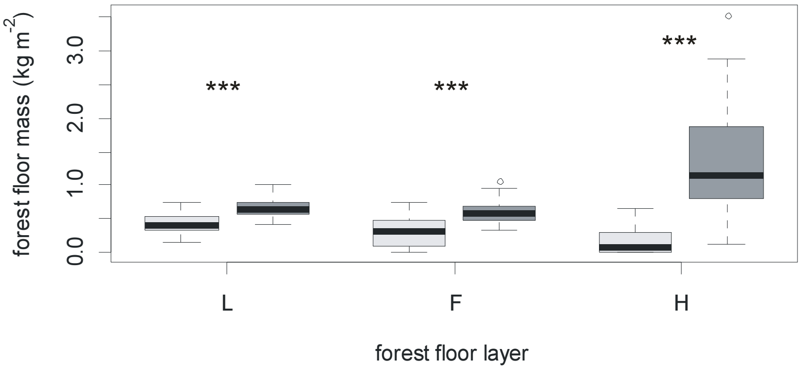


Figure 3.2. Comparison of the biomass of the forest floor layers (L: litter layer; F: fragmentation layer; H: humus layer) of the deciduous (light grey) and spruce (dark grey) stands. Significant differences are shown (Wilcoxon signed-rank test): * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

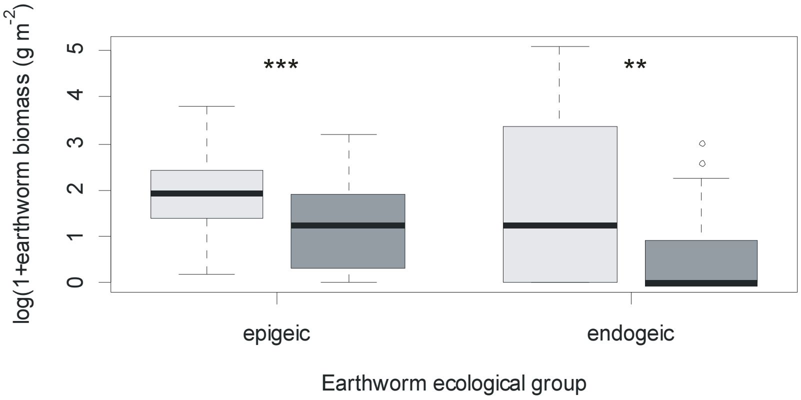


Figure 3.3. Comparison of the epigeic and endogeic earthworm biomass of the deciduous (light grey) and spruce (dark grey) stands. Significant differences are shown (Wilcoxon signed-rank test): * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

3.4). For the total forest floor mass, differences between deciduous and coniferous stands could not be related to the soil buffering gradient, although the fragmentation layer difference was correlated with the clay gradient (rs 0.73; n = 40; $P < 0.0001$). The differences in total earthworm biomass within a deciduous-spruce pair increased with increasing clay content. An even clearer correlation between the logarithm of the earthworm biomass and pH-KCl was found: the difference increased significantly with higher pH-KCl in the deciduous stands (Figure 3.5).

3.4 Discussion

We will discuss the changes we observed along a natural soil gradient as a consequence of deciduous forest conversion to spruce. We focused our analysis on changes in 1) different soil acidification variables, 2) forest floor characteristics, and 3) earthworm communities. First we check if the sampling design was valid by discussing the clay gradient and by verifying whether the soil conditions were comparable within pairs along the gradient. Second, we evaluate the general effects of the conversion on the mentioned variables. Third we evaluate if the large gradient in potential soil buffering capacity - clay contents varied between 3 and 34 % - had an effect on the rate of changes after conversion, and fourth we try to explain the mechanisms behind the observed differences in change trajectory along the buffering gradient.

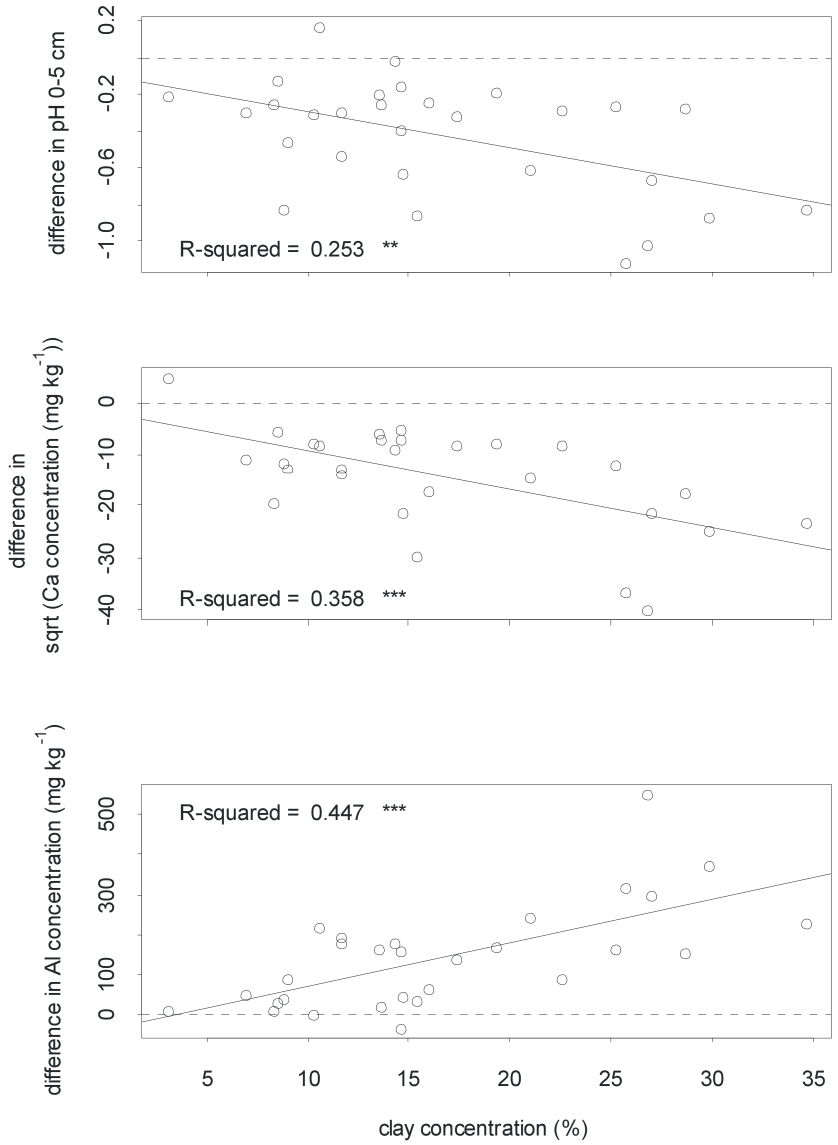


Figure 3.4. Relation between the clay content of the soil as proxy of the soil buffering gradient and the difference in pH, calcium and aluminium concentrations in the topsoil (0 – 5 cm) within a deciduous-spruce pair. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

3.4.1 Was the sampling design satisfactory to answer our research questions?

Based on the soil texture, and more specifically the clay content, we assessed the gradient in soil buffering capacity of our sites. Mineral weathering is buffering the soil at any pH and the buffering rate is depending on the weathering rate. Its buffering capacity is generally large depending on the soil type, especially the clay-size fraction. Clay content is also known to determine, among others, the cation exchange capacity of soils. Clay particles and organic material tend to be negatively charged and hold cations through electrostatic forces. Both determine the cation exchange capacity (CEC); this is the total

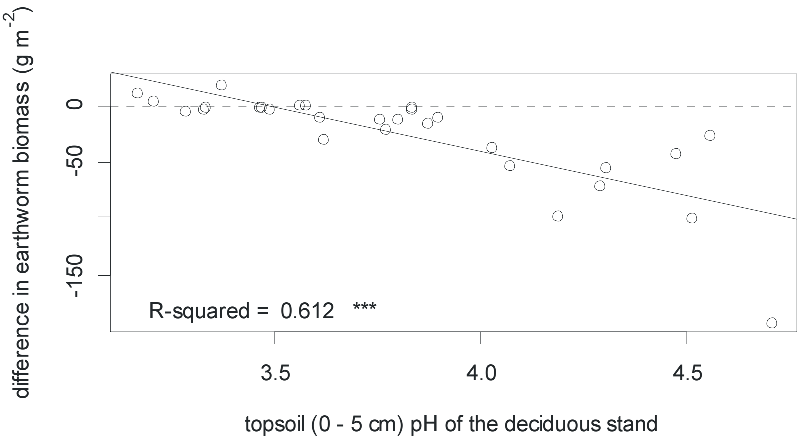


Figure 3.5. Relation between topsoil (0-5 cm) pH-KCl of the deciduous stands and the difference in earthworm biomass within a deciduous-spruce pair. Regression is done without the plot with highest pH and biggest difference between the stands. Significance: *** $P \leq 0.001$.

of the exchangeable cations that a soil can hold at a specified pH. Soils with a higher CEC have a higher buffering capacity and are able to buffer more acidifying input. The CEC range of clay minerals is usually between 10 to 150 cmol kg^{-1}, while the CEC of organic matter may range from 200 to 400 cmol kg^{-1} depending on the pH (Parker, 2010). Differences in the content and type of clay and organic matter in a soil create large variation in CEC between soils. The percentage of organic matter decreases in the deeper soil layers and higher clay content of a soil compared to the organic matter content makes the clay content important for determining the CEC, and thus also the buffering capacity of the soil. The clay content of the soil is not influenced by the vegetation. Here, the soils of the deciduous stands were in the cation exchange buffer or in the transition zone to the aluminium buffer range. Therefore, we could use the clay content of the soil as a proxy of the initial buffering capacity of the soil, independent of the vegetation. The initial soil buffering gradient determined the potential tree species composition across the sites. In the study region, well-buffered soils generally facilitate tree species with rapid decomposing leaf litter (Verstraeten *et al.*, 2013b). Leaf litter with high calcium concentrations and low C:N ratios exhibits higher decomposition rates and can improve the chemical properties of the topsoil (Reich *et al.*, 2005; Hobbie *et al.*, 2006; De Schrijver *et al.*, 2012). The initial gradient of soil buffering capacity interacts with the tree species composition to determine the current soil buffering capacity, soil pH and calcium concentrations. Also several other variables were related with the clay content, such as the forest floor mass, the earthworm community and the understorey vegetation composition varied along the gradient.

Pairwise comparison of the soil acidification variables in the deciduous and converted spruce stands showed no differences for pH and calcium concentration in the deepest soil layer sampled (25 – 35 cm). The influence of tree species on chemical soil parameters in the deeper soil layer is considered to be smaller for the deeper soil layers as showed by studies of post-agriculture afforestations with different tree species (Hagen-Thorn *et al.*, 2004; De Schrijver *et al.*, 2012). The similarity of pH and Ca content in deepest soil layers sampled strengthens our hypothesis, together with the *ex ante* verification of historic information and *in situ* verification of the soil profile, that the situation before conversion was equal in both stands. In the deepest soil layer, there was a difference in Al concentrations, probably due to the leaching of Al from the more shallow soils layers. Other parameters, e.g. climate and nitrogen and sulphur concentrations in the air, are similar for both stands in a pair during the years. Therefore, we could assume that the measured differences between deciduous and spruce stands can largely be attributed to different tree species composition.

3.4.2 What was the overall effect of conversion from ancient deciduous to spruce stands in terms of soil acidification variables, forest floor characteristics and earthworm communities?

The overstorey, the soil acidification variables and the earthworm biomass in the deciduous stands were significantly related after a long period of continued forest cover (a stable state *sensu* Scheffer *et al.*, 2001). However, large changes, such as the conversion to a tree species with a different chemical composition of the leaf litter, could have large impacts on the whole system. The conversion of ancient deciduous stands into spruce stands highly impacted on the variables related to acidification in the topsoil layer, on the forest floor mass and on the earthworm community. Spruce is a strongly acidifying tree species compared with most of the deciduous species (Ranger and Nys, 1994; Augusto *et al.*, 1998; Binkley and Giardina, 1998; Reich *et al.*, 2005). After conversion, the chemical variables of the upper 20 cm of the mineral soil shifted towards more acid conditions. This is in accordance with several other observational and experimental studies where the pH and the base cation pools are lower and the acid cation pools higher under spruce trees (e.g. Tamm and Hallbäcken, 1986; Augusto *et al.*, 1998; Hagen-Thorn *et al.*, 2004). Differences between tree species in variables concerning acidification are more significant in the upper soil layers (De Schrijver *et al.*, 2012). In this study, we also found a smaller acidification with increasing depth. We did not find any correlation with the age of the stands and the change trajectories, probably because the small age difference between the stands (most stands were planted within a timespan of 15 year).

The forest floor mass was found to be significantly higher in the spruce stands compared to the deciduous forest matrix and there was an accumulation of organic matter in the H-layer. It is known that lower calcium concentrations and higher C:N ratio concentrations of spruce trees lower the decomposition rate (Augusto *et al.*, 2002; Reich *et al.*, 2005; Hobbie *et al.*, 2006). The lower decomposition rate of spruce leaf litter and the decreased bioturbation by earthworms probably caused the forest floor build-up, especially in the H-layer. This forest floor build-up could acidify the soil through the production of organic acids as intermediate decomposition products (de Vries and Breeuwsma, 1985) and by causing a time delay in the return of buffering cations to the soil where they were taken up by tree roots in exchange for protons (Nilsson *et al.*, 1982).

The earthworm biomass declines when soil pH and calcium concentrations decrease (Nordstrøm and Rundgren, 1974; Edwards, 2004) and also with decreasing litter quality. Especially lower calcium concentrations and high C:N in leaf litter, will lower earthworm biomass (Reich *et al.*, 2005; Hobbie *et al.*, 2006). The total earthworm biomass decreased after conversion to spruce. Earthworms fragment freshly fallen litter and drag it deeper into the organic and mineral soil through their burrows, leading to increased organic matter turnover rates (Muys *et al.*, 1992). Higher forest floor mass was significantly related with lower earthworm biomass (Figure 3.6). Muys and Granval (1997) proposed that the presence of endogeic earthworms is a good indicator that soils are in the cation exchange buffer range. Endogeic earthworm species disappeared in the deciduous stands on less buffered soils (pH-KCl < 3.5; also an indicative value of the transition to the aluminium buffer range) and in nearly all spruce stands because of their sensitivity for acidification. However, these endogeic species are important to maintain a mull humus type due to their important bioturbation activity (Muys and Granval, 1997). Epigeic species lived along the whole gradient, but their biomass decreased after conversion to spruce.

3.4.3 Are differences in change trajectory between the deciduous and spruce stands related to the soil buffering capacity?

Change trajectories after conversion were larger on the well buffered soils for soil properties related to acidification, for the F layer of the forest floor and for the earthworm biomass. The absence of a relation between the clay content of the soil and the difference within a deciduous-spruce pair in total forest floor mass is probably due to the big difference between both stand types: almost half of the deciduous stands had no H-layer while in spruce stands the litter accumulates in the H-layer and the total forest floor mass was significantly higher in a spruce plot than its adjacent deciduous plot. For the other variables, we assume that the following three interrelated causes importantly contributed to the spruce-deciduous difference along the gradient in buffer capacity: the difference in buffer capacity, the shifts in the earthworm community and the differences in litter quality. (i) The soil can buffer incoming protons with a series of buffer reactions along the soil pH-gradient (Bowman *et al.*, 2008). The capacity of a soil to buffer incoming protons depends on the buffer system: the drop in soil pH for one unit acidifying input will be larger in the base cation exchange buffer range (indicative interval: pH-KCl between 4.3 and 3.5 sensu Bowman *et al.*, 2008) - especially at the lower pH-values of this interval - than in the hydroxyl-aluminium buffer range (pH-KCl < 3.5). The cation exchange buffer range is a small buffer which is very quickly exhausted in situations where large proton input occurs. Since we can assume that the acidifying input in the spruce stands was similar along the gradient of soil buffering capacity, the difference in topsoil pH is expected to be larger at the well buffered sites in the cation exchange buffer range. (ii) We further assume that the increased earthworm mass and presence of endogeic earthworm species at the well-buffered sites contributed to the larger differences between deciduous and coniferous stands on these sites. Endogeic species will disappear when soil reach the aluminium buffer range. On the more acid sites, we found no endogeic earthworms in both stand types and the differences in biomass within these pairs were small. On the well buffered soils, endogeic earthworms were numerously found in the deciduous stands, while we could not detect them in the spruce stands. This caused the bigger difference in earthworm mass within a pair on the well buffered soils. This bigger difference in earthworm biomass could also enhance differences within pairs for soil variables and the forest floor because of the importance of earthworms in maintaining good humus quality and more general as ecosystem engineer (e.g. Jones *et al.*, 1994; Barot *et al.*, 2007). (iii) The composition of the deciduous forest matrix changed along the buffering gradient: on the well-buffered sites occurred more species with fast decomposing litter (such as *F. excelsior*, *P. avium, Acer* spp.) while the sites with low buffering capacity were the stands dominated by oak and beech. The difference in leaf litter quality between oak or beech and spruce is smaller than between species with good litter quality like *F. excelsior* en *P. avium* and spruce (Hobbie *et al.*, 2006; Vesterdal *et al.*, 2008). This makes that the disturbance driver is larger and the effects on the soil, forest floor and earthworm community are also larger on the well buffered sites.

3.4.4 Interconnectedness between tree species, soil, forest floor and earthworm community

In Figure 3.6, we showed an overview diagram of deciduous and spruce stands together and try to explain the relations between the four important variables measured in this study: tree species, soil, forest floor and earthworm community. De Schrijver *et al.* (2012) described relations between these and other variables and we adapted that conceptual model to our situation and our measured variables. All variables were closely interrelated and relations could be bidirectional. As described above, the tree species composition and the soil buffering capacity (with clay content used as proxy) interact as important factors determining the biogeochemical state of the forest system. The variable *tree composition * clay* is negatively correlated with the forest floor (Figure 3.6). Forest floor build-up increased in plots on less buffered sites and with lower leaf litter quality. Poor leaf litter quality and low buffering capacity contributes also to a lower earthworm biomass which result in retarded leaf litter decomposition and lower burrowing activity and consequently in forest floor build-up. Earthworm biomass was also significantly correlated with soil acidification because earthworms, and especially burrowing species, accelerate the base cation circulation in the soil (De Schrijver *et al.*, 2012). Forest floor build-up resulted also in soil acidification as described already higher in the discussion. The variable *tree composition * clay* is also significantly correlated with topsoil pH because the clay content determines the initial soil buffering capacity and several direct and indirect effects related to tree species composition influence the soil pH.

The strong relations between the different variables studied demonstrated their complex interactions. It is clear that conversion to spruce had a significant impact on the abiotic and biotic state of the forest. Change trajectories of soil variables concerning acidification and earthworms were longer on the initial well buffered soils. Interconnectedness

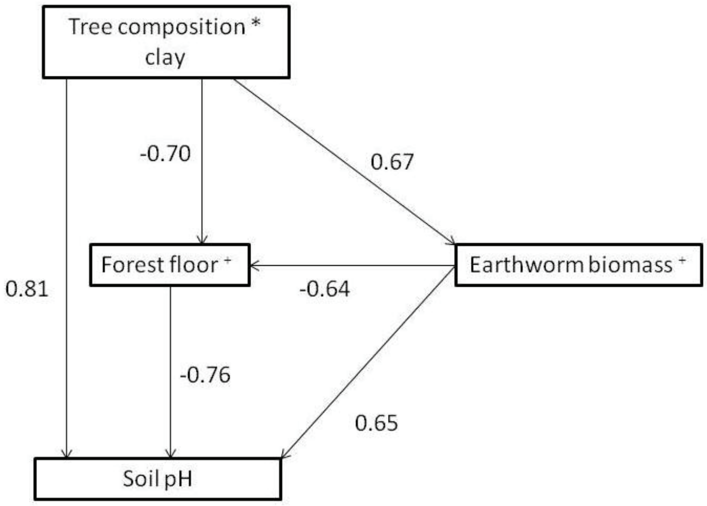


Figure 3.6: Synthesis diagram with standardised regression coefficients showing the relation between: tree composition * clay, forest floor (total forest floor mass), earthworm biomass (both epigeic and endogeic earthworm species) and soil pH-KCl. All regressions were significant ($P \leq 0.001$). + variable is logarithmic transformed.

between the factors and as a consequence the positive feedback loops likely enhance the effects of the conversions. After 35 to 50 years of conversion to spruce, the shifts are clearly visible for several variables of the forest system but it is unclear if this state is already at a new equilibrium. The system made already a large shift from a mesotrophic to an oligotrophic state but the continuous pressure of high acidifying input in the spruce stands compared to the deciduous stands could possibly cause further acidification.

Deciduous and coniferous forest, Fammene (Belgium), 2013

4

Understorey vegetation shifts following the conversion of temperate deciduous forest to spruce plantation

After: Verstraeten G., Baeten L., De Frenne P., Vanhellemont M., Thomaes A., Boonen W., Muys B. & Verheyen K. (2013). Understorey vegetation shifts following the conversion of temperate deciduous forest to spruce plantation. Forest Ecology and Management, 289, 363-370.

Abstract

The identity and composition of the overstorey trees can have large impacts on the understorey vegetation in temperate forests. Here, we assess the effects of the conversion of mixed deciduous forests to Norway spruce (*Picea abies*) plantations on the understorey vegetation composition and diversity. We also investigated whether the effects of this conversion depend on the soil buffering capacity and if they affect the species pool at the landscape scale. The study area contained ancient deciduous woodlands mainly consisting of *Quercus* spp., *Fagus sylvatica* and *Carpinus betulus* in the Gaume, an area with a mild temperate climate in southern Belgium. Spruce plantations were established 35–50 years ago in this matrix of deciduous woodlands. Differences in overstorey and understorey vegetation, forest floor and soil characteristics between 40 paired plots in spruce plantations and adjacent deciduous forest were compared along a gradient of soil buffering capacity. The forest floor mass was higher and the soil pH was lower in spruce plantations. The composition of the understorey vegetation differed between deciduous forest and spruce plantations: the spruce stands contained more light-demanding and acid-tolerant understorey species than the adjacent deciduous stands. The mean understorey species diversity, i.e., the local species diversity in a plot (α-diversity) as well as the total number of species (γ-diversity), did not differ significantly between the deciduous and spruce stands. Hence, the differing species composition of the spruce plantations contributes to a larger overall species pool and higher across site β-diversity. Although the differences in the environmental variables within a plot pair increased along the gradient of soil buffering capacity, the differences in understorey vegetation diversity between deciduous and spruce stands showed no relationship with the gradient. Only the difference in Ellenberg indicator value for soil reaction (R) within a pair

increased with increasing soil soil buffering capacity. In line with the abiotic changes, the understorey vegetation composition had shifted towards a more acid-tolerant vegetation type 35–50 years after conversion to spruce plantations. The increase in light-demanding species was probably due to a more intensive thinning management in the spruce stands.

4.1 Introduction

As a reaction to the global loss of biodiversity, biological conservation ranks high on the policy agendas (e.g., Millenium Ecosystem Assessment 2005). In forestry, the interest in biodiversity conservation has also increased, and the conservation of biodiversity became an important goal of multifunctional and sustainable forest management (e.g., Ministerial Conference of the Protection of Forest in Europe 1993). The understorey vegetation contributes substantially to the total biodiversity of temperate forests, although it makes up only a small proportion of the total forest biomass (Gilliam, 2007). The understorey diversity and composition is profoundly influenced by the species composition of the tree canopy (Hunter, 1999; Gilliam, 2007; Barbier *et al.*, 2008) and by site conditions such as soil and climate characteristics (Augusto *et al.*, 2003). The overstorey directly affects the understorey through its impact on light availability (Härdtle *et al.*, 2003; Tinya *et al.*, 2009) and by competition for water and nutrients (Small and McCarthy, 2005; Van Calster *et al.*, 2008). Trees also have indirect effects on the understorey, especially through the quality and quantity of their litter, which influences the chemical soil conditions (Augusto *et al.*, 2002; Van Calster *et al.*, 2007; Mölder *et al.*, 2008; Thomaes *et al.*, 2012), the soil fauna (Reich *et al.*, 2005) and the recruitment and growing conditions of the vegetation (Sydes and Grime, 1981; Verheyen *et al.*, 2003b; Baeten *et al.*, 2009a). As litter quality and quantity differ among tree species, the species composition of the overstorey layer is expected to influence the understorey. Yet, the effects of tree diversity on the understorey vary from study to study (Barbier *et al.*, 2008), for instance because canopy diversity is often confounded with management practices and site conditions. More recently however, Mölder *et al.* (2008) and Vockenhuber *et al.* (2011) showed that the diversity of the understorey layer is higher in forest stands with high tree diversity because of their higher environmental heterogeneity.

For economic reasons, the tree species composition of many forests in western and central Europe has been changed since the beginning of the 20th century. Large areas of mixed deciduous forests were converted into conifer plantations (Spiecker *et al.*, 2004), and, more recently, reconverted to mixed or deciduous forests (Spiecker *et al.*, 2004;

Ammer *et al.*, 2008). The ecological impacts of those conversions are an important topic for biodiversity conservation. Here, we focus on the conversion of traditionally managed, semi-natural deciduous forests mainly consisting of oak (*Quercus* spp.), hornbeam (*Carpinus betulus*) and beech (*Fagus sylvatica*) towards plantations with non-native, coniferous tree species (Norway spruce *Picea abies,* further called spruce). As such, we could study the influence of forest conversion on the understorey vegetation composition and diversity and whether these changes depend on the local soil conditions. We looked already to the temporal changes in the vegetation (Chapter 2) and found clear differences between the two site types, both in the composition and the temporal changes. Therefore, we expect also differences after conversion from deciduous to spruce along the gradient of soil buffering capacity. We took advantage of an unintentional experimental setting formed by patches of spruce plantation in a large mixed deciduous ancient forest. To our knowledge, the effect of a specific conversion type (e.g., from mixed deciduous to spruce monoculture) along a relatively long soil gradient was never studied in a comparable manner.

4.2 Materials and methods

4.2.1 Study site

The study area was a part of a large forest complex in the south of Belgium (Gaume region, described in chapter 1.3.1).

4.2.2 Study design and data collection

To determine how forest conversion from mixed deciduous forest into spruce plantation affected the understorey community, we compared 40 pairs of unconverted (deciduous) and adjacent converted (spruce) stands along a gradient of soil buffering capacity. The plots were selected in a step-wise approach: the first selection based on topographic maps was refined in the field.

Firstly, all the spruce stands in the study region were identified on aerial photographs and topographical maps; stands at the outer edges of the forest complex were excluded. Only stands of 35-50 years old were retained because these stands were in the tree stage and therefore comparable with the deciduous stands. The soil texture map of the Service Public de Wallonie (2007) and the vegetation map of Dethioux and Vanden Berghen (1966) were used to choose plots distributed along a broad gradient of soil buffering capacity. The soil texture (especially the clay content of the soil) was used to determine the initial site richness gradient, i.e., independent of vegetation influences. The initial gradient determined the potential tree species composition across the sites. In the study region, well buffered sites generally facilitate tree species with rapid decomposing leaf litter (Verstraeten *et al*. 2013b). Leaf litter with high nutrient concentrations and low C and lignin values and, consequently, also high C:N ratios, exhibits higher decomposition rates and can improve the chemical properties of the topsoil (Reich *et al.*, 2005; Hobbie *et al.*, 2006; De Schrijver *et al.*, 2012). Thus, the initial soil buffering gradient interacts with the tree species composition and both variables are important determinants of the current soil buffering gradient (Appendix 1).

Secondly, the preselected spruce and adjacent deciduous stands were examined in the field. Paired deciduous and spruce stands ought to have similar elevation, slope and slope orientation. Stands that were recently clearcut were omitted. An important assumption in this study design was that, just before planting the spruce, both stands were comparable in forest history, tree species composition, vegetation and abiotic variables. This was controlled *ex ante* by comparison of maps and management archives, by *in situ* comparison of soil profiles and by *ex post* comparison of the chemical soil properties of the deepest soil layer sampled (25-35 cm).

The centre of the plots was always 30 m from the border into both the deciduous and coniferous stand. We chose the distance of 30 m from the border (about one tree height) to minimize plot differences as well as mutual influences among the stands. In June 2009, the vascular plant community was recorded in squared plots of 10 m × 10 m in each of the 40 selected paired stands, separately for the herbaceous (< 1 m, including brambles and ferns), shrub (> 1 m and < 7 m) and tree layer (> 7 m). Recordings were made using a decimal cover scale (Londo 1976) and back-transformed to percent cover values.

Soil variables were determined by analysing soil characteristics at three depth intervals (0 – 5 cm, 10 – 20 cm and 25 – 35 cm). Soil samples were randomly collected within each quadrant of the plot and for each depth interval pooled into a composite soil sample. The soil samples were dried at 40°C until constant weight and analysed for pH (extraction in 1 M KCl, 1:5 suspension, ion-specific electrode) at the three depths and Ca^{2+} and Al^{3+} (extraction in 0.1 M $BaCl_2$, 1:20 suspension, Dutch standard method NEN 5738; flame atomic absorption spectrophotometry, SpectrAA-220, Varian) for the 0 – 5 cm and 25 –

35 cm layer. The soil texture of the 10 – 20 cm depth interval (expressed in percentages clay, sand and loam) was determined by laser granulometry (Malvern Mastersizer S). The forest floor (comprising the litter, fragmentation and humus layer) was collected in four squares of 20 cm x 20 cm in each plot. These samples were dried at 70°C until constant weight and weighted.

4.2.3 Data analysis

The data analysis consists of three major parts: we analysed the differences between the deciduous and spruce plots for the abiotic variables (1) and the understorey community diversity (2) and composition (3). The differences within a deciduous – spruce pair for these variables were considered along the gradient of soil buffering capacity. We used the clay concentration of the soil as an indicator of the initial soil gradient of soil buffering capacity and the topsoil pH of the deciduous stands as an indicator of the current gradient of soil buffering capacity. All differences between deciduous and spruce stands were tested with the Wilcoxon signed-rank test.

The understorey diversity was evaluated at three spatial scales. (1) At the plot level (α-diversity), two indices were used: the total number of species in a plot (species richness) and the Shannon-Wiener diversity (cover-weighted α-diversity index). (2) The γ-diversity for the two forest types was the number of all species occurring in the deciduous or spruce stands. (3) The dissimilarity in floristic composition (β-diversity) was quantified with the Lennon dissimilarity index (Lennon *et al.*, 2001). This index is calculated as $\min(b, c)/[\min(b,c)+a]$, with '*a*' representing the number of species shared by both plots; '*b*' the number of species that occur in the deciduous plot only and '*c*' the number of species unique to the spruce plot. The mean dissimilarity of a plot against all other plots within the same stand type was calculated as that plot's community divergence, and the dissimilarities were compared between deciduous and coniferous stands. Furthermore, the compositional turnover within each plot pair was calculated using the Lennon index.

Compositional differences between the two stand types were tested with a permutational analysis of variance (PerMANOVA, Anderson, 2001; McArdle and Anderson, 2001). We used Bray-Curtis dissimilarities and 999 permutations for testing the differences in composition. Next, we focussed on the species and species characteristics that underlie the compositional differences to gain more insight into the factors that mainly determine the community differentiation. We first identified the species that occur in higher frequencies or a higher cover in one of the stand types using indicator species analysis (Dufrêne and Legendre, 1997). Then we calculated the Ellenberg indicator values to assess the underlying environmental causes of the differences in composition between the deciduous and spruce stands. The Ellenberg indicator values (Ellenberg and Leuschner, 2010) are plant-derived variables related to the prevailing environmental conditions. For this study, we used the Ellenberg values that express individual species' requirement for light (L), soil reaction or acidity (R), nitrogen state of the soil (N, often more generally interpreted as soil fertility) and soil moisture (F). Ellenberg average indicator values (AIV) were then calculated for each plot as unweighted averages of the indicator values of the understorey species occurring in that plot. Because we expected an effect of the deciduous vs. evergreen canopy, we determined the differences in the flowering period of the understorey (based on flowering period of understorey species specified in De Langhe *et al.*, 1995).

4.3 Results

4.3.1 Differences in soil and forest floor

The topsoil pH (0-5 cm) was significantly higher in the deciduous stands compared with the spruce stands (Figure 4.1). The pH of the soil layer from 25 to 35 cm did not differ between the two stand types. The exchangeable Al^{3+} concentrations were higher and the exchangeable Ca^{2+} concentrations were lower in spruce plantations compared with deciduous stands (Table 4.1). The differences within a deciduous - spruce plot pair for pH, exchangeable Ca^{2+} and Al^{3+} concentrations were correlated with the gradient in soil buffering capacity; the differences increased towards the well buffered sites (Figure 4.2). The forest floor mass was significantly higher in spruce stands (Table 4.1).

4.3.2 Differences in understorey diversity

We recorded 108 vascular plant species in the understorey layer across the 40 paired deciduous-spruce plots. The species pool (γ-diversity) of both stand types was exactly the same, i.e., 86 understorey species occurred in both the deciduous and the spruce stands; 64 of the species were found in both stand types, 22 species occurred only in the deciduous stands, and 22 were confined to the spruce stands.

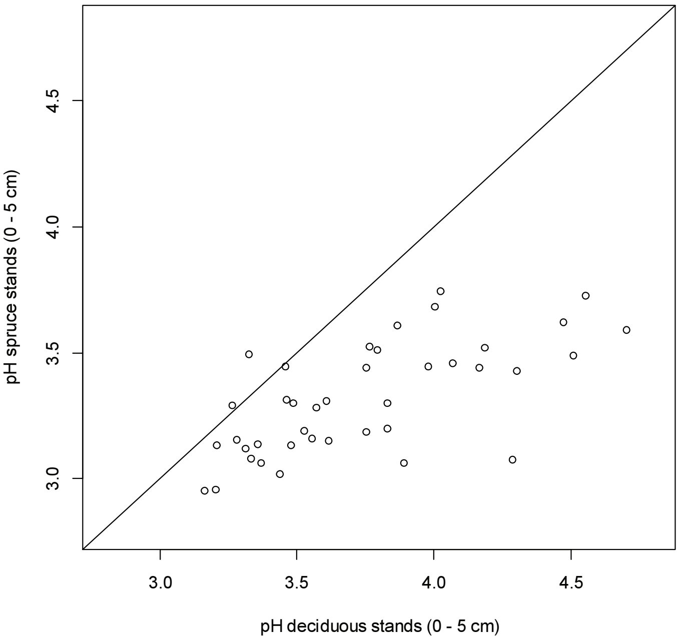


Figure 4.1: Topsoil pH (0 – 5 cm) in the 40 paired plots in deciduous forest and adjacent spruce plantations. The full line is the 1:1 line of no difference; points below the 1:1 line indicate that the topsoil pH of the deciduous stands is higher than in the spruce stands.

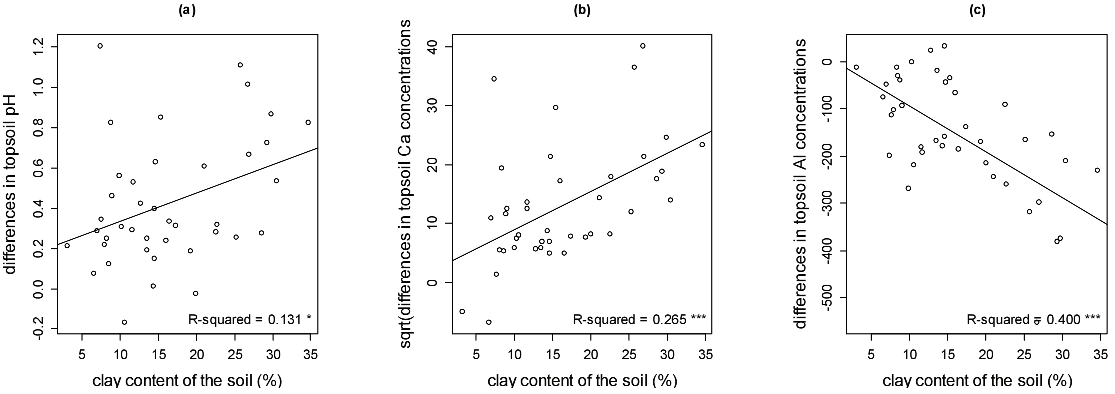


Figure 4.2. Differences between deciduous and spruce stands for topsoil pH (0 – 5 cm) (a), Ca concentrations (b) and Al concentrations (c) along the gradient of soil buffering capacity (clay content of the soil). Significance: NS not significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Table 4.1. Characteristics (median values and between brackets the 0.25 and 0.75 quantiles values) of the soil and forest floor in the deciduous and spruce stands.

	Deciduous stands	**Spruce stands**	**Test-statistic *W***	***P* value**
Soil				
pH				
0 – 5 cm	3.7 [3,4 – 4.0]	3.3 [3.1 – 3.5]	1309.5	< 0.001
25 – 35 cm	4.2 [4.1 – 4.5]	4.2 [4.0 – 4.3]	925	0.231
Exchangeable Al^{3+} concentration (mg/kg)				
0 – 5 cm	119.5 [60.0 – 170.0]	308.5 [164.5 – 346.5]	272.5	< 0.001
25 – 35 cm	86.0 [51.0 – 120.0]	138.5 [76.0 – 231.5]	484.5	0.003
Exchangeable Ca^{2+} concentration (mg/kg)				
0 – 5 cm	186.0 [105.8 – 503.2]	73.9 [52.4 – 118.1]	1309	< 0.001
25 – 35 cm	14.9 [8.5 – 200.5]	8.2 [5.8 – 89.0]	1005	0.049
Forest Floor				
Forest floor mass (g/m^2)	837.2 [622.1 – 1292.3]	2355.1 [1914.7 – 3076.7]	13	<0.001

The species diversity in a plot (α-diversity) was equal for both stand types, i.e., the median was 19 species per plot (Table 4.2). Yet, the Shannon-Wiener diversity index was significantly higher in the spruce stands. The significantly higher Shannon-Wiener diversity index together with the equal number of species indicates that the evenness in cover among the species was higher in the spruce stands. The mean cover of the understorey layer was significantly higher in the deciduous plots than in the coniferous plots (Table 4.2). The species richness of a plot increased with topsoil pH for both stand types, with a steeper increase in the spruce stands (Figure 4.3a), and with increasing clay content in the soil (Figure 4.3b). The differences in species richness within a plot pair were not related to the clay content of the soil (Figure 4.4a). The Lennon dissimilarity index (β-diversity) of the deciduous and spruce stands did not significantly differ (Table 4.2). So, the mean compositional differences within the deciduous plots were equal to the mean differences within the spruce plots.

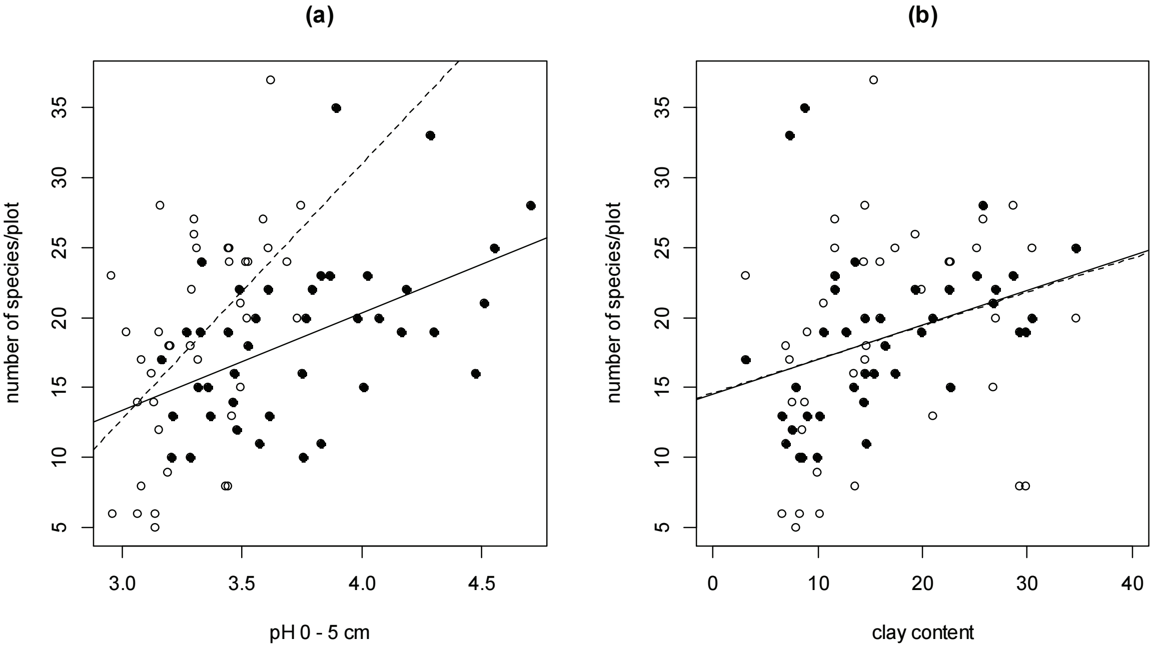


Figure 4.3: Species richness in relation to the topsoil pH (a) and the clay content (b). Filled and open circles represent plots in deciduous and spruce stands, respectively. The solid line shows the linear regression for the deciduous stands (pH 0-5 cm: $R^2 = 0.276$, n = 40, P < 0.001; clay content: $R^2 = 0.119$, n = 40, P = 0.029); the dotted line shows the linear regressions for the spruce stands (pH 0-5 cm: $R^2 = 0.26$, n = 40, P < 0.001; clay content: $R^2 = 0.065$, n = 40, P = 0.040)

Table 4.2. Characteristics (median values and between brackets the 0.25 and 0.75 quantiles values) of the vascular understorey vegetation in the deciduous and spruce stands.

	Deciduous stands	Spruce Stands	Test-statistic *W*	*P* value
α-diversity				
Species number/plot	19 [14.5 – 22]	19 [13.5 – 24.5]	840	0.707
Shannon-Wiener diversity	1.63 [1.18 – 1.91]	2.29 [1.79 – 2.65]	1254	< 0.001
β-diversity				
Lennon dissimilarity	0.54 [0.49 – 0.58]	0.58 [0.52 – 0.61]	975	0.093
γ-diversity				
Species pool	86	86		
Cover				
Cover (%)	77 [40 – 114]	38 [11 – 61]	369.5	< 0.001
Ellenberg indicator values				
Light (AIV_L)	4.43 [4.05 – 4.72]	4.79 [4.47 – 5.00]	1179	< 0.001
Moisture (AIV_F)	5.24 [5.06 – 5.40]	5.31 [5.06 – 5.53]	916	0.265
Soil reaction (AIV_R)	5.21 [4.05 – 6.25]	4.24 [3.61 – 4.91]	420	< 0.001
Nitrogen (AIV_N)	5.42 [4.83 – 6.08]	5.18 [4.78 – 5.75]	687	0.297

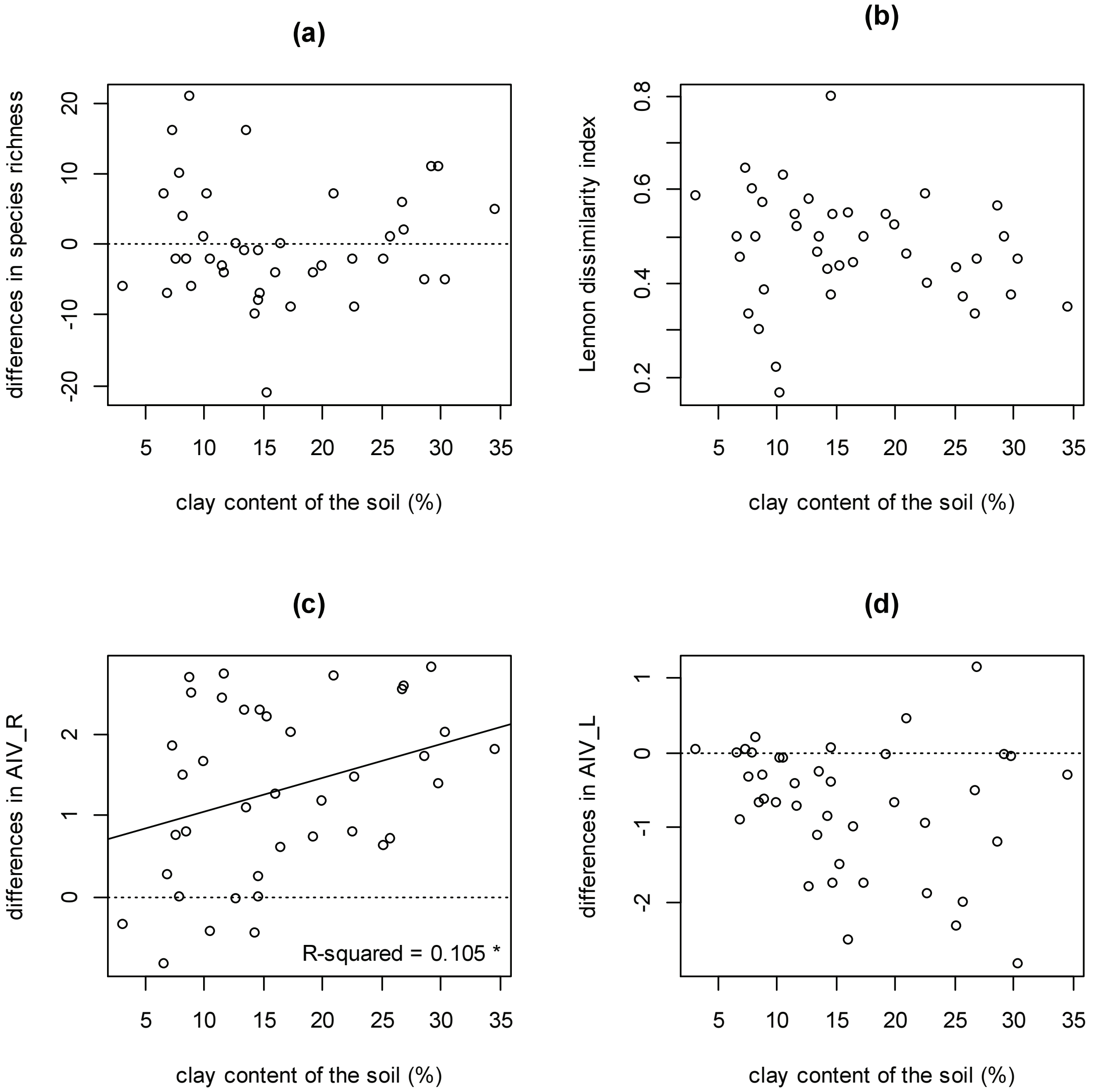


Figure 4.4. Differences between the paired plots in deciduous forest and spruce plantations along the gradient of soil buffering capacity: species richness (a), Lennon dissimilarity (b), AIV_R Average Ellenberg indicator value for soil reaction (c), AIV_L Average Ellenberg indicator value for light (c). If the relationship was significant, the regression line was shown. * P < 0.05

4.3.3 Differences in understorey composition

The species composition differed significantly between deciduous and spruce stands (PerManova, P < 0.001). The dissimilarity between the two plots of a pair was not correlated with the topsoil gradient of soil buffering capacity of the deciduous stands (Figure 4.4b). The indicator species analysis showed more forbs in the deciduous stands and more ferns and graminoids in the spruce stands (Table 4.3). The indicator species of the spruce stands were species from a more acid soil type (cf. *Hypericum pulchrum, Luzula luzuloides, Carex pilulifera*) and species that need more light (cf. *Digitalis purpurea* and *Silene dioica*). The mean Ellenberg indicator values for the plots were consistent with the indicator species analysis: AIV_R was significantly lower and AIV_L significantly higher in the spruce stands (Table 4.2). Hence, the vegetation in the spruce plots consisted of more acid-tolerant and light-demanding understorey plant species. The difference in AIV_R within a deciduous-spruce pair increased significantly with increasing soil clay content (Figure 4.4c), while there was no relation between the AIV_L and the clay content (Figure 4.4d). The flowering period of understorey vegetation in deciduous and spruce stands differed significantly: the peak of flowering was earlier for plants in the deciduous stands (Figure 4.5).

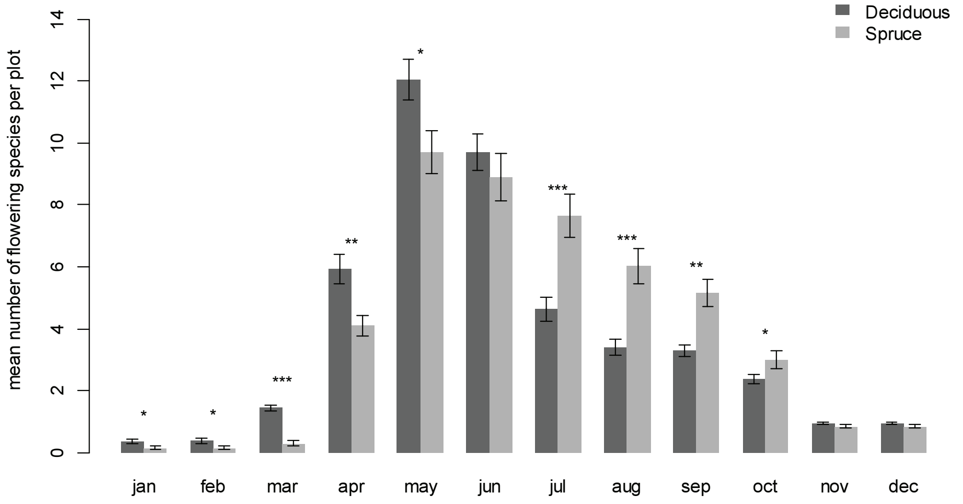


Figure 4.5. The mean number of flowering understorey species per plot in each month of the year. Error bars represent standard errors. Significance of the difference in flowering understorey species between deciduous and spruce stands:* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

Table 4.3. Species occurring in the 40 paired plots in deciduous forest and spruce plantations that are significantly associated (in terms of occurrence and/or cover) with deciduous versus spruce stands, determined by an indicator species analysis. Between the parentheses, the percentage of the plots were the species occurs (n=40) is shown. Species marked with * are ancient forest species, according to Hermy *et al*. (1999), or species with a recovery rate smaller than -0.5, according to De Frenne *et al*. (2011). De Frenne *et al*. (2011) quantified the recovery rate (RR) of species as the log-response ratio of the percentage occurrence in post-agricultural over ancient forests across 18 European landscapes. Species with an RR smaller than -0.5 (i.e., with the percentage occurrence in ancient forests approximately 1.65 times the percentage in post-agricultural forests) were classified as ancient forest species.

	Deciduous stands	**Spruce stands**
Forbs	*Adoxa moschatellina* * (25)	*Hypericum pulchrum* * (15)
	Anemone nemorosa * (100)	*Oxalis acetosella* * (45)
	Convallaria majalis * (57.5)	*Veronica officinalis* * (20)
	*Stellaria holostea** (42.5)	*Moehringia trinervia* * (47.5)
	Galium odoratum * (35)	*Epilobium montanum* * (20)
	Lamiastrum galeobdolon * (47.5)	*Digitalis purpurea* * (35)
	Maianthemum bifolium * (40)	*Mycelis muralis* (15)
	Paris quadrifolia *(30)	*Silene dioica* (30)
	Polygonatum multiflorum * (82.5)	*Rubus idaeus* (72.5)
	Arum maculatum * (12.5)	
	Lonicera periclymenum * (22.5)	
	Stachys sylvatica * (27.5)	
	Geum urbanum (15)	
	Alliaria petiolata (15)	
Tree seedlings	*Acer platanoides* (35)	*Sambucus racemosa* (37.5)
	Fagus sylvatica (47.5)	*Picea abies* (90)
	Fraxinus excelsior (35)	
Graminoids		*Luzula pilosa* * (50)
		Luzula luzuloides * (75)
		Carex pilulifera * (32.5)
		Festuca altissima (25)
		Juncus effusus (22.5)
Ferns		*Dryopteris carthusiana* * (82.5)
		Dryopteris dilatata (60)

4.4 Discussion

The soil, forest floor and understorey community composition of the first-generation spruce plantations differed considerably from the paired deciduous forest stands consisting of native tree species. The conversion of traditionally managed, deciduous forest to spruce plantations seems to have had a significant impact on the soil, forest floor and understorey vegetation with a general shift towards a more acido-tolerant type. Soil acidification, and the associated changes in exchangeable calcium and aluminium concentrations, first occur in the topsoil layer; the initial effects on the deeper soil layers are smaller (De Schrijver *et al*., 2012). Indeed, the soil pH of the deepest soil layer did not differ within a plot pair (Table 4.1), which strengthens our assumption that the soil conditions of the selected plot pairs were similar before the spruce trees were planted. The calcium and aluminium concentrations of the deepest soil layer, however, did significantly differ within a pair (Table 4.1), but the differences were much smaller than in the topsoil.

Many conifer tree species tend to acidify soils more than deciduous tree species, mainly due to the poor litter quality (low leaf nutrient concentrations and high C and lignin concentrations) and the higher interception of atmospheric pollutants (Binkley and Giardina, 1998; Augusto *et al.*, 2003; Reich *et al.*, 2005; De Schrijver *et al.*, 2007). A poor leaf litter quality decreases the rates of litter decomposition and the presence of burrowing earthworms, which will increase the build-up of the forest floor and result in soil acidification (De Schrijver *et al*., 2012). The calcium concentration of leaf litter is an important determinant of litter decomposition rates in the presence of earthworms.

Lignin-poor and nitrogen-rich leaves exhibit higher decomposition rates in systems with few earthworms (Reich *et al.,* 2005; Hobbie *et al*., 2006). Differences in the capacity to capture potentially acidifying and eutrophying deposition among tree species are also an important cause of soil acidification (De Schrijver *et al*., 2012). Spruce has a higher interception capacity than deciduous species mainly due to its evergreen character (Erisman and Draaijers, 2003). All these factors together explain the acidification potential of the conversion of native deciduous into spruce forests.

The clay content of the soil was an important independent determinant of the gradient of soil buffering capacity in this study. Along the gradient of increasing soil buffering capacity, the differences within a deciduous-spruce plot pair increased for several soil variables and for the forest floor mass. This pattern can be explained by the changes in the soil buffer regime with increasing acidifying input (Bowman *et al.*, 2008). The drop in soil pH for one unit acidifying input will be larger in the base cation exchange buffer range (pH_{KCl} between 7.0 and 3.5) - especially at the lower pH-values of this interval - than in the hydroxyl-aluminium buffer range ($pH_{KCl} < 3.5$). If we assume that the acidifying input in the spruce stands was similar along the gradient of soil buffering capacity, the difference in topsoil pH will be larger at the well buffered sites. The differences within a pair for several soil variables can be further enhanced by the higher mean litter quality on the well buffered sites (Verstraeten *et al.*, 2013b). The contrast between litter quality of spruce and deciduous tree species is expected to increase along the gradient of soil buffering capacity.

Given that the forest floor and the topsoil determine the physico-chemical growth conditions for understorey plants, changing their chemical and physical properties will influence the understorey vegetation. The acidification of the topsoil and accumulation of litter due to the conversion to spruce in the study area were reflected in the changes towards a more acid-tolerant vegetation. The increased differences in abiotic soil variables within a pair along the gradient of soil buffering capacity were also reflected in the species composition, with bigger differences for AIV_R on the richer sites. In addition, the spruce understorey also contained more light-demanding species. Light is a crucial resource that often limits the growth of forest understorey species (Bazzaz, 1979; Kirby, 1988; Jennings *et al.*, 1999). Light transmittance through spruce canopies is commonly considered as lower than in deciduous species (Augusto *et al.*, 2002). However, the variation among different deciduous tree species is high (Canham *et al.*, 1994; Ellenberg and Leuschner, 2010) and the forest management often determines the light climate to a greater extent (Cutini, 1996; Augusto *et al.*, 2003). In our study area, however, more light is transmitted through the spruce canopies in summer, mainly due to the more intensive thinning management in these stands. Plant species that cannot survive in the darker deciduous stands seem to have established in the spruce stands. Several of these species are characteristic of open habitats and forest edges (e.g., *Digitalis purpurea, Silene dioica*). The higher cover and frequency of graminoids in the spruce stands may also be caused by a higher understorey light availability (Decocq *et al.*, 2004). Since spruce is an evergreen species, the changes in light conditions throughout the year will differ in the spruce stands compared to the deciduous stands. The light availability for the understorey vegetation will be higher in the leafless deciduous stands than in the coniferous stands, which is probably the cause for the earlier flowering peak of the understorey species in the deciduous stands. Early, spring-flowering forest understorey species have a typical shade-avoiding strategy; they use the brief period before the leafing out of the trees for photosynthesis (Schemske *et al.*, 1978). Species such as *Anemone nemorosa* and *Adoxa moschatellina* were growing in many of the plots in the deciduous stands, but could probably not survive in the spruce stands due to a lack of light in early spring. There was no relation between the gradient of soil buffering capacity and the difference in AIV_L within a pair. Next to the influence on the understorey vegetation composition, light is often assumed to be the major limiting resource for understorey cover (Barbier *et al.*, 2008). Yet, in our study, the mean understorey cover was higher in the darker deciduous stands, which may be due to the higher light levels in deciduous stands in spring and the more acid soil and thicker forest floor in spruce stands.

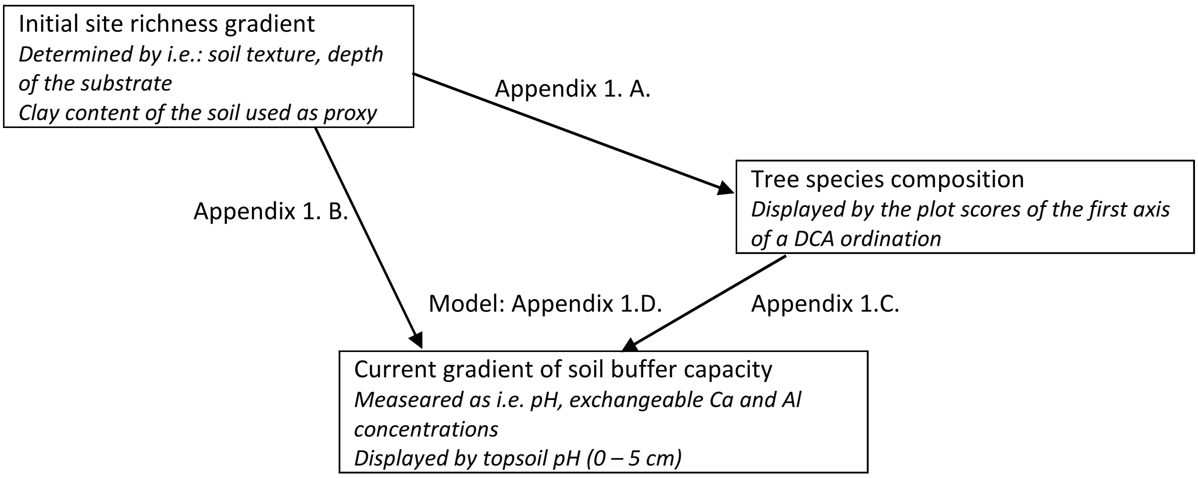
The light regime and soil acidity seem the two most important variables to explain the differences in species composition between deciduous and spruce stands. Yet, additional factors may also be relevant to explain these differences. Firstly, the average soil moisture level by spruce stands is frequently considered to be lower than in deciduous stands (Augusto *et al*., 2002). The higher interception of water in spruce canopies decreases the water amount that reaches the forest floor. Stand variables such as the lower stand density and single vertical structure class also increase water throughfall (Carlyle-Moses *et al.*, 2004; Levia and Frost, 2006). In our study, however, the Ellenberg F values did not indicate differences in soil water availability between the deciduous and spruce plots. Secondly, the disturbances during forest conversion, i.e., the harvest of the deciduous trees and the planting of the spruce trees, can destroy the vegetation. Several forest understorey species are slowly-colonising species (e.g. *Convallaria majalis* and *Galium odoratum*; De Frenne *et al*., 2011) and will not be able to colonise the spruce stands in the short time period since harvesting, even if the environmental circumstances are suitable for the species. Thirdly, the morphological differences between coniferous and deciduous litter and the thicker forest floor in spruce stands may have had a differential influence on the understorey layer. The forest floor can hamper seed germination and seedling establishment of understorey plants (Eriksson, 1995). Many understorey plant species are well adapted to penetrate deciduous litter with their shoots, but may experience difficulties to grow in the thick forest floor of spruce.

While the vegetation composition shifted following the forest conversion, the understorey diversity did not change significantly. In a meta-analysis comparing the vascular understorey diversity in several deciduous and coniferous stands, Barbier *et al.* (2008) identified five studies with higher understorey species richness in coniferous and nine studies with higher species richness in deciduous stands. Differences in species diversity seem to depend on the local abiotic circumstances and management. In our study area, the spruce stands were managed in such a way that they have a lower canopy cover than the deciduous stands. The higher light availability in the spruce stands probably compensated their lower soil pH, which stabilized the species richness over time. Similarly, Augusto *et al.* (2003) found a higher understorey species richness in thinned spruce stands than in deciduous stands or unthinned spruce stands. On the other hand, soil pH is positively correlated with species diversity in many Central-European forests (Brunet *et al.*, 1997; Ewald, 2003; Schuster and Diekmann, 2003; Partel *et al.*, 2004; Kooijman, 2010), and also in our study region, species richness generally increases with soil pH (Verstraeten *et al.*, 2013b). We expected to find a relation between the gradient of soil buffering capacity and the difference in understorey diversity within a plot pair because the chemical topsoil characteristics showed larger differences on the well buffered sites than on the poor sites. However, no relationship between the gradient of soil buffering capacity and the differences in species richness and dissimilarity within pairs was found.

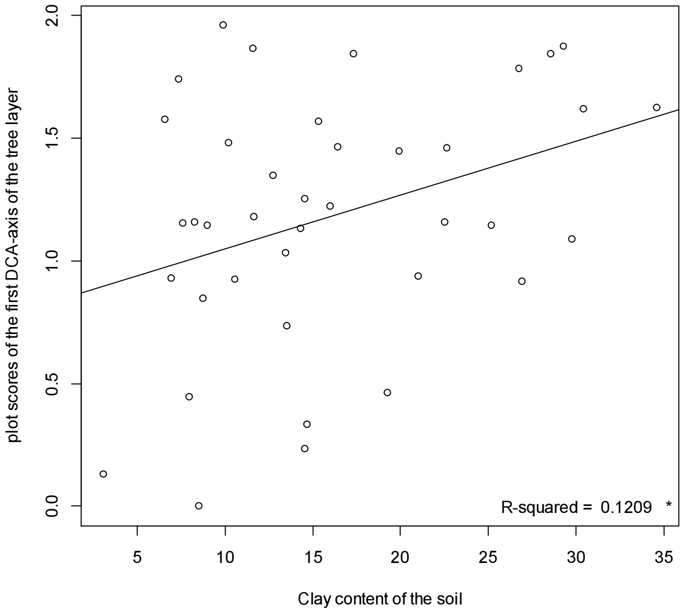
To conclude, the understorey communities in the deciduous and coniferous stands were similar in terms of their local species richness and compositional variation, but their species composition differed considerably. The islands of spruce plantations in the deciduous forest matrix provided a larger overall species pool and a higher across-site β-diversity, increasing the biodiversity value at the scale of our study region. The conversion to spruce plantations was done especially from an economic interest. We showed that the impact on the understorey diversity of the small patches was rather limited. However, there are some important comments to be made from a conservation point of view. Firstly, all the coniferous stands in the present study were first-generation spruce stands. The abiotic variables already showed a significant shift towards more oligotrophic conditions, but the present situation is likely to be an intermediate state. We do expect a further acidification of the sites due to the permanently high acidification potential of the spruce trees. Acidification might enhance species loss in the longer term in the spruce stands because species richness generally decreases with a decreasing soil pH. Secondly, many of the recorded understorey species probably survived in the spruce plots because of the enhanced light availability. These relatively light-demanding species also occur in open habitats and at other places in the deciduous forest and forest edges, and are less interesting from a biodiversity conservation point-of-view.

4.5 Appendix 1

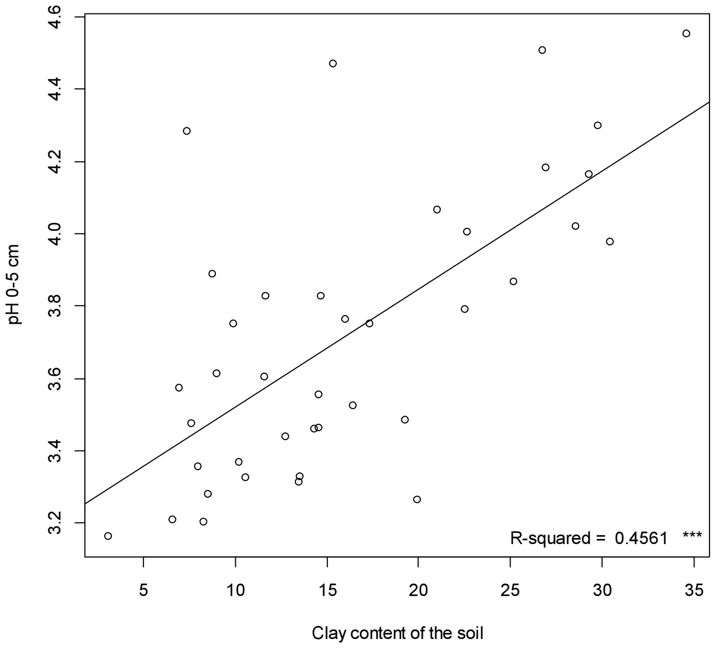
Simplified conceptual model explaining the relations between initial site richness gradient, the tree species composition and the current gradient of soil buffering capacity:



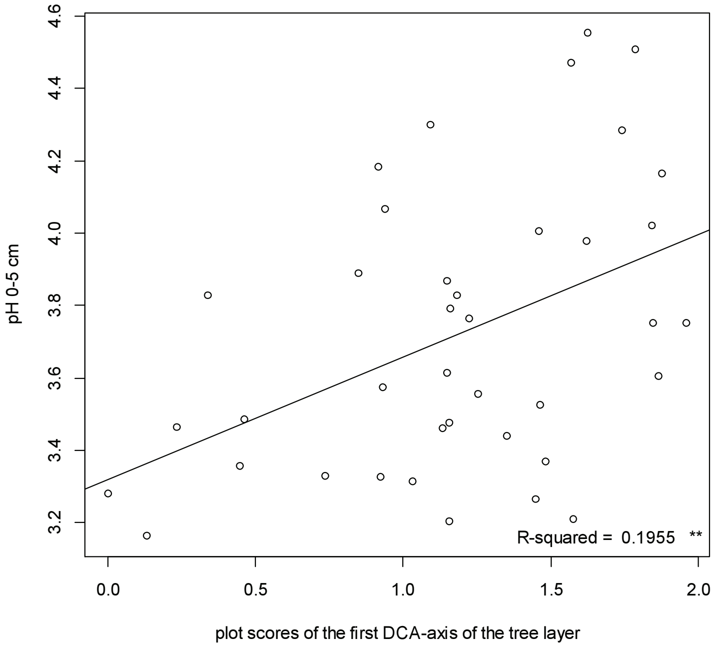
A. Correlation between initial site richness and tree species composition



B. Correlation between initial site richness and current gradient of soil buffering capacity



C. Correlation between tree species composition and current gradient of soil buffering capacity



D. Model results

Model: *current site richness ~ initial site richness + tree species composition*

lm(formula = *pH 0-5 cm ~ clay content of the soil+ plot scores of the first axis of a DCA ordination*)

	value	*P*
Intercept	23.621	< 2e-16
plot scores of the first axis of a DCA ordination	1.886	0.067
clay content of the soil	0.0286	3.27e-05

Box 2: Changes in the seed bank after conversion from deciduous forest to spruce plantations

The vegetation changed significantly after conversion. So, we also expected changes in the seed bank after conversion from deciduous forest to spruce plantations. Furthermore, the soil seed bank could give us additional information about the restoration potential after conversion: some species not found in the present-day understorey vegetation layer may still be present in the seed bank. In this box, we present the results of a study that compared the soil seed bank under deciduous forest and adjacent spruce plantations in our study area (see also Sluijs, 2012).

This study was done in a subset of twenty out of the forty studied plots in chapter 4. The twenty plots were selected along the whole gradient in soil buffering capacity. The field survey was done in August 2011. We relocated the 10 m x 10 m plots used in chapter 3 and 4 and made a new vegetation survey. In each of these plots a 2 m × 2 m plot was placed in the centre and this 4m² plot was subdivided into 25 0.4 m × 0.4 m plots. Strings were used to visualize the grid in the field and seed bank samples (0 - 5 cm and 5 – 10 cm deep, Ø = 3.4 cm) were collected systematically on the grid nodes (36 samples). The concentrated seed-bank samples (Ter Heerdt *et al*., 1996) were spread out over potting soil and placed in a greenhouse under a 16 hour day – 8 hour night regime with daytime temperatures between 20 and 25°C. The samples were kept moist through capillary rise and were drained by gravity. The germination period started in September and was terminated after two consecutive weeks of no new seedling records, 23 weeks after the start of the germination experiment. No additional chilling treatment was applied. All identified seedlings were counted and removed, whilst unidentified seedlings were transplanted and brought to flower for later identification.

A total 26460 seeds germinated, belonging to 50 different species. The median number of germinated seeds was as double as high in the spruce stands compared to the deciduous stands (Table Box 2.1). The majority of the seedlings were *Juncus effusus* (80%) and this species occurred in nearly all the plots (Figure Box 2.2). Without the seeds of *J. effusus*, the difference in number of germinated seeds between the deciduous and the spruce stands was not significant, nor was there any difference in species richness. All species found in spruce plots were also found in deciduous plots. Two species were only found in deciduous plots, these species were ancient forest species. However, the number of germinated species was significantly correlated with the topsoil pH gradient: the difference was larger on the soils with higher pH in the deciduous stand (Figure Box 2.1).

We calculated the average Ellenberg indicator values (AIV) for light (L), nitrogen (N) and soil reaction (R) and found significant lower AIV_R values in the spruce plantations (Table Box 2.1). The germinated species in the seed bank of spruce plantations were more acid-tolerant compared to the species in the deciduous stands.

The results of the study showed that the differences in the seed bank after conversion are in line with the results of the understorey vegetation: there where no differences in median species richness per plot, the species were more acid-tolerant in the spruce plantations and the differences between the deciduous and spruce stands were larger at de moderately acidic sites. In contrast with the results for the understorey vegetation, there were no differences between the deciduous and spruce stands in the light requirements of the seed bank species.

Only one species significantly associated with deciduous plots versus seven species significantly associated with spruce stands (Table 4.3) were found back in more than three seed bank plots (Figure Box 2.2) . The understorey species associated with spruce were not more frequent in the seed bank in spruce; only two out of the seven species appeared in the seed bank of more spruce plots than deciduous plots. The presence in the understorey of *Stachys sylvatica* was significantly associated with deciduous plots, and in the seed bank, the species also only appeared in deciduous plots. Hence, most indicators of deciduous understorey plots were not retrieved in the seed bank of both the deciduous and spruce stands.

Table Box 2.1. Characteristics (median values and the 0.25 and 0.75 quantiles values between brackets) for germinated species in the deciduous and spruce stands (Wilcoxon signed rank-sum test). Significance: NS, not significant; (*) P ≤ 0.10; * P ≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001.

	Deciduous	spruce	test-statistic *V*	Significance
Number of germinated species	11 [9 - 12.25]	11.5 [10 - 13.25]	56	NS
Number of germinated seeds				
All seedlings (# m^{-2})	3364 [2410 - 15624]	6123 [3247 - 42416]	47	*
Without *J. effusus* (# m^{-2})	2480 [2232 - 4309]	3658 [1627.5 - 6138]	83	NS
Average Ellenberg indicator values (AIV)				
Light (AIV_L)	5.56 [5.33 - 6.00]	5.68 [5.47 - 6.00]	90	NS
Soil reaction (AIV_R)	4.76 [4.13 - 5.19]	4.40 [4.08 - 4.67]	183	***
Nitrogen (AIV_N)	5.07 [4.84 - 5.67]	4.95 [4.74 - 5.17]	145	NS

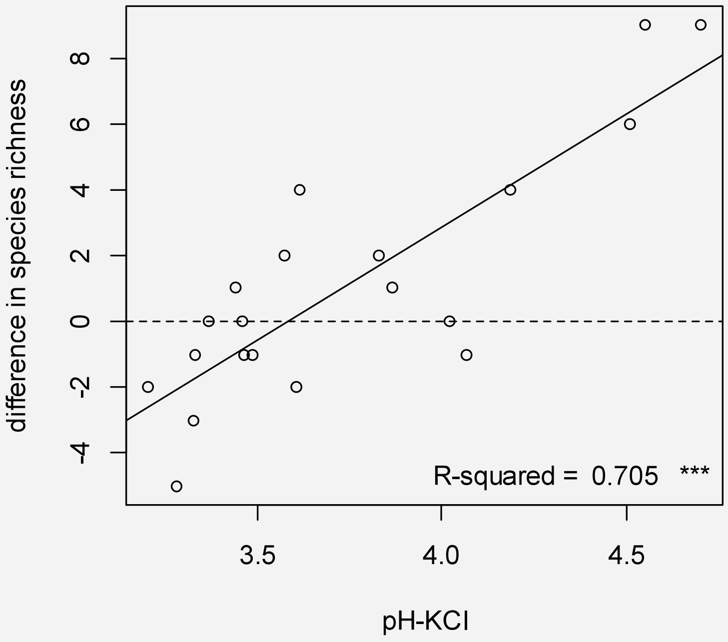


Figure Box 2.1. Relation between topsoil (0 – 5 cm) pH-KCl of the deciduous stands and the difference in number of germinated species within a deciduous-spruce pair [number of species in spruce – number of species in deciduous].

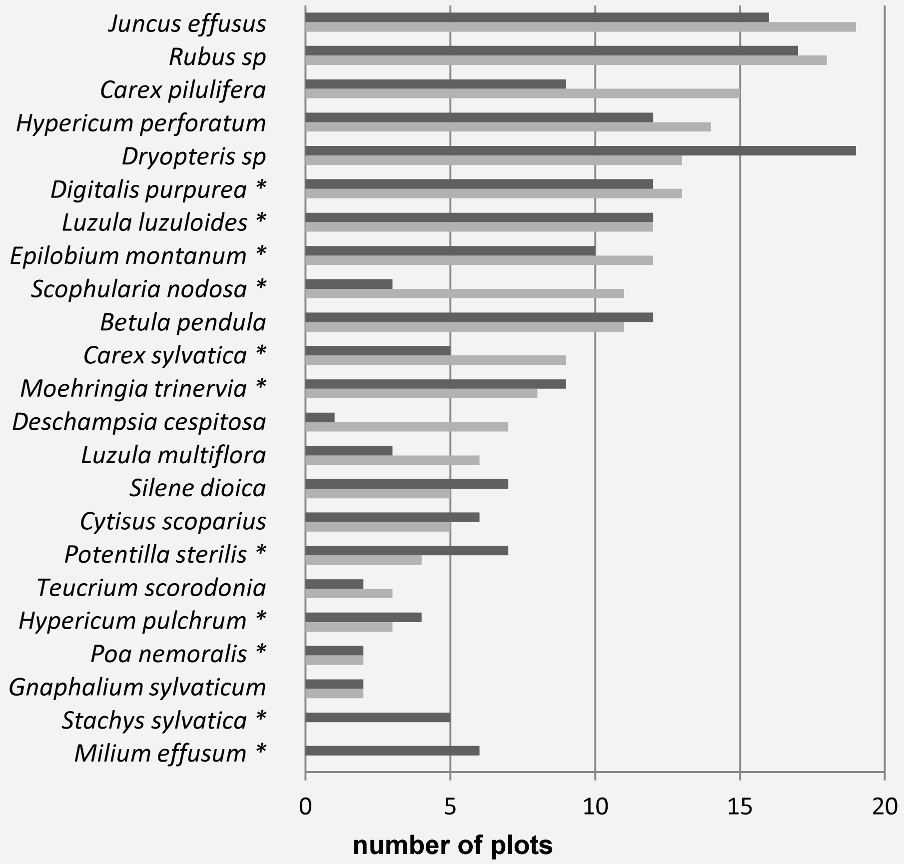


Figure Box 2.2. Overview of the species in the seed bank of deciduous forest (dark grey) and spruce plantations (light grey). Only species occurring in more than three plots are shown. * are ancient forest species, according to Hermy *et al*. (1999), or species with a recovery rate smaller than -0.5, according to De Frenne *et al*. (2011). De Frenne *et al*. (2011) quantified the recovery rate (RR) of species as the log-response ratio of the percentage occurrence in post-agricultural over ancient forests across 18 European landscapes. Species with an RR smaller than -0.5 (i.e., with the percentage occurrence in ancient forests approximately 1.65 times the percentage in post-agricultural forests) were classified as ancient forest species.

Primula elatior

5

Forest herbs show species-specific responses to the variation in light regime and soil acidity: an experiment mimicking forest conversion scenarios

After: Verstraeten G., Baeten L., De Frenne P., Thomaes A., Demey A., Muys B. & Verheyen K. Forest herbs show species-specific responses to the variation in light regime and soil acidity: an experiment mimicking forest conversion scenarios. Basic and Applied Ecology, s*ubmitted.*

Abstract

Forest conversion from native deciduous forests to coniferous stands has been performed in many European regions and resulted in dramatic shifts in understorey plant community composition. However, the drivers for changes in specific understorey plant species remain unclear.

Here, we experimentally determine the species-specific effects of two important ecological factors that are known to change as a consequence of conversion, i.e. the light availability and chemical soil characteristics, on the vegetative and regenerative performance of five characteristic herbaceous forest understorey plants. Topsoil samples from both spruce and deciduous stands at four locations, with two levels of soil acidity, were collected and used in a common garden experiment. Additionally, three different light levels were applied, i.e., 'light deciduous', 'dark deciduous' (extra light reduction during summer) and 'evergreen' (light reduction during winter). In a second experiment we evaluated the germination of two of these species against the site type and tree species at the site of origin of the soil samples.

The light regime was an important factor affecting both the vegetative and regenerative performance of the understorey species: compared to light deciduous, *Anemone nemorosa* had a significantly lower performance under the evergreen light regime, *Convallaria majalis* under dark deciduous and *Luzula luzuloides* and *Galium odoratum* under both light regimes. The vegetative performance was lower in soil from acid sites for the acid-sensitive species *G. odoratum* and *Primula elatior*. Differences in the soil sampled under deciduous or spruce stands had no effect on the vegetative, nor regenerative performance of these species. By

contrast, the germination of *L. luzuloides* and *P. elatior* was higher in soils sampled in deciduous stands and in neutral sites.

Species-specific responses in vegetative and regenerative performance of adult plants to a changed light regime and soil acidification could be a reason for the changed vegetation composition in converted stands. Also lower germination and establishment of forest understorey species in spruce stands could influence the species distribution after conversion.

5.1 Introduction

The composition of the regional pool of forest plant species is principally controlled by climatological, geographical and geological characteristics (Augusto *et al.*, 2003). At the local scale, the composition of the forest tree canopy, among other factors, plays an extremely important role in determining the understorey species diversity and community composition (e.g. van Oijen *et al.*, 2005; Barbier *et al.*, 2008; Wulf and Naaf, 2009). The ecological characteristics of the tree species and their abundance affect local environmental characteristics important for understorey species, including the amount of light transmittance (Barbier *et al.*, 2008) and the leaf litter quality and quantity influencing several biotic and abiotic soil properties (Reich *et al.*, 2005; Kooijman, 2010; De Schrijver *et al.*, 2012). Therefore, changes in the composition of canopy tree species can significantly impact the local understorey species diversity and composition of forests.

During the 19th and 20th century, large areas of native deciduous forests in Western and Central Europe were converted to coniferous plantations, mainly motivated by an economic interest (Spiecker *et al.*, 2004). The conversion from native deciduous, broadleaved temperate forests to Norway spruce (*Picea abies*, further referred to as "spruce") plantations is for instance widespread and changed chemical soil characteristics, the forest floor environment and the understorey composition in many European regions (e.g. Ewald, 2000; Máliš *et al.*, 2012; Verstraeten *et al.*, 2013a). Coniferous tree species contribute significantly to the soil acidification, often more than many deciduous tree species (Augusto *et al.*, 1998; Augusto *et al.*, 2002), and also change the understorey light regime. Lower light transmittance is particularly evident in winter due to their evergreen character. Nevertheless, the often more frequent thinning in coniferous stands creates gaps in the canopy which causes a higher, and probably also more heterogeneous, light availability at the forest floor. The changes in soil, litter and light conditions may cause changes in the understorey community towards more acid-tolerant species with higher light requirements in coniferous stands (e.g. Verstraeten *et al.*, 2013a).

Several similar observational studies stressed already the effects of changes in tree species composition on the understorey layer (e.g. Ewald, 2000; Van Calster *et al.*, 2007; Mölder *et al.*, 2008; Wulf and Naaf, 2009; Kooijman, 2010), yet they mainly reported community-level patterns of change. The influence of tree species conversion on species-specific adult plant performance and germination, on the other hand, are more difficult to study in the field and therefore less explored. Pot-experiments allow testing individual species responses to particular environmental factors such as soil acidity (Falkengren-Grerup and Tyler, 1993; Tyler, 1996), tree species effects (Thomaes *et al.*, 2011) and light availability (Baeten *et al.*, 2010b). However, the combination of light regime and acidification and the effect of an evergreen versus deciduous light regime have not been studied yet.

Widespread conversions of native deciduous forests to coniferous plantations over the past decades have led to significant changes in the forest understorey communities of those forests. In this experiment we tested how the altered light regime and soil conditions after conversion might have influenced plant performance. We examined the response of five characteristic herbaceous understorey plant species to variations in the light regime (evergreen vs. light and dark deciduous) and tree species induced changes of soil characteristics (using soil sampled in deciduous and spruce forest). Because the impact of deciduous forest conversion to spruce on the soil conditions might depend on inherent site conditions (Verstraeten *et al.*, 2013a), we also included a site type treatment using soil from sites with two levels of soil acidity. We selected five forest understorey species that are known to respond to forest conversion and that are different in terms of their leaf and flowering phenology and sensitivity to soil acidification. The vegetative and regenerative performance of each of these species was measured in a pot experiment. Moreover, the recruitment in terms of their germination of two of these species was also determined. We hypothesized to find species-specific changes in vegetative and regenerative performance in response to a changed light and soil conditions. Our first hypothesis is that the flowering period and the leaf phenology of a species will affect the response of the species performance on light regime. Spring-flowering species will be more sensitive for light availability in spring while summer-flowering species for the light regime in summer. The second hypothesis is the response to differences in soil conditions will depend on the sensitivity of the species for soil acidification, depending on their occurrence in field. We also expected to find lower germination in soil from spruce stands from acid sites.

5.2 Materials and Methods

5.2.1 Species selection and experimental design

We selected five herbaceous forest species (*Anemone nemorosa*, *Convallaria majalis*, *Galium odoratum*, *Luzula luzuloides* and *Primula elatior*) that are common in forests in Western Europe (Table 5.1). The selected species showed a strong change in frequency and abundances as a consequence of conversion from deciduous forests to spruce plantations in an observational study by Verstraeten *et al.* (2013a), see Table 5.1. The species were also selected to represent different plant traits in terms of sensitivity for acidity and different leaf and flowering phenologies (Table 5.1). The vegetative and regenerative performance of these species as a function of soil and light regime was tested in a common garden. Plants were retrieved from a commercial nursery specialised in wild plants where individuals or ramets (for *A. nemorosa*) of the five studied species were grown in optimal conditions from seeds or rhizomes in small pots (0.5 L) filled with standard potting soil. The location of origin of the seeds and ramets is unknown. For each species, the seeds were selected at one location and were grown up in similar conditions. In March 2010, healthy plants of the five species were transplanted in larger containers (2.4 L) with forest soil from forest stands in the south of Belgium. All potting soil was washed off the roots with water and no extra nutrients were added (Baeten *et al.*, 2010b).

To assess the effect of site quality, tree species and light regime on the plant performance, we performed a full factorial experiment. We selected two sites with acid soil conditions and two sites with more neutral soil conditions (factor *site type*, levels: acid and neutral site) located in the south of Belgium (49.7 °N, 5.5 °E). The selection of the four sites was based on soil data (pH, calcium and aluminium concentrations and clay content) of the observational study (Verstraeten *et al.*, 2013a). The *tree species* treatment was introduced by taking soil from a deciduous stand (dominant tree species *Quercus robur, Fagus sylvatica* and *Carpinus betulus*) and the adjacent first generation spruce stand at each site – we assume the latter presented similar soil conditions prior to the spruce planting (~

four decades ago; Verstraeten *et al*. 2013a). With this design, we could specifically focus on the variation in plant performance caused by differences in chemical soil variables due to difference in tree species (factor *tree species*, levels: soil from spruce and deciduous stand). The soil was collected by removing the litter layer and excavating the upper mineral soil layer (0 – 5 cm) and was thoroughly mixed to a homogeneous sample. We only took the topsoil layer because tree species effects are most significant in the topsoil (De Schrijver *et al.*, 2012; Verstraeten *et al.*, 2013a) and forest understorey species are rooting mainly in the topsoil. Chemical soil analyses confirmed the differences in soil properties and showed that the topsoil (0 – 5 cm) pH and calcium concentrations were lower and the aluminium concentrations were higher at the locations on the acid sites compared to the locations on the neutral sites. The mean pH-KCl for soil sampled in the deciduous and the spruce stands was 3.57 and 3.23, respectively, for the most acidic sites and 4.63 and 3.53 for the less acidic sites.

After transplantation, the pots were put out in the same homogeneous forest conditions for the rest of the growing season to recover from transplantation stress (Baeten *et al.*, 2010b). The pots were placed in a homogeneous *Fraxinus excelsior* (tree layer) dominated forest stand of a forest near Ghent, Belgium (51.0 °N, 3.8 °E). In the autumn of 2010, we started with three different light treatments, each applied to three experimental plots (N = 9 plots). In each plot, we placed one replicate of each '*site* x *tree species*' combination for every forest understorey plant species (2 tree species × 4 sites × 5 understorey species: $N = 40$ in each plot). The three light regimes were: light deciduous, dark deciduous and evergreen (factor *light regime*). The plots were placed in a relatively open deciduous stand, so no extra shading was given for light regime "light deciduous". To simulate the "dark deciduous" light regime, plots were covered with shade cloth from canopy flush in spring till leaf shedding in autumn (third week of April until the end of October). The polyethylene shade cloth was placed at 110 cm height (70% light reduction; Baeten *et al.*, 2010b). The light regime "evergreen" was based on the light regime in the relatively open spruce stands: the light transmittance is comparable with a light deciduous stands in summer, but this canopy shade is also present in winter. To simulate the evergreen light regime, the shade cloths were installed from the moment of leaf shedding in autumn till the moment of canopy flush in spring (end of October until the third week of April).

We determined several aspects of the performance of plants by measuring a suite of species-specific traits related to growth and reproduction (cf. Endels *et al.*, 2004; Baeten *et al.*, 2009b; Baeten *et al.*, 2010b). Table 5.1 gives an overview of the measured vegetative and regenerative traits for each species. The different morphology of the species required us to measure different traits on the five species. All measurements were done in the second year after planting, just following the peak flowering time of each species. For the regenerative traits of *C. majalis* we used data from the first growing season (2011) because there were no flowering plants of this species in 2012.

5.2.2 Seed germination experiment

The second experiment was started for all the species except *C. majalis* because of low seed availability for this species. Seeds of *A. nemorosa* were collected in a forest stand near our common garden site at seed maturity in 2010 and the seeds of the three other species were retrieved from a commercial nursery specialised in wild plants with local origin. Seeds of *A. nemorosa* were stored in moist sand at room temperature because of the risk of seed desiccation (Ali *et al.*, 2007) and seeds of the other species were kept air dry at room temperature. Small pots (17 cm³) were filled with soil from the two more acidic and two less acidic sites and for each site sampled in a deciduous and spruce stand (similar as above, factor *site type* and *tree species*). We put 10 seeds in each pot and had 10 replicates for each combination of site and tree species (2 *tree species* * 4 sites * 10 replicates ~ $N = 80$ for each species). The pots were placed in a growth chamber where optimal conditions for germination were simulated. The pots were randomly rotated in the growth chamber each week and distilled water was supplied regularly. Based on earlier studies (Baskin and Baskin, 1998) seeds were given 10 weeks of warm stratification (14h: 20 °C & full light; 10h: 10 °C & dark), 10 weeks with intermediate temperature (12h: 12 °C & full light; 12h: 6 °C & dark) and 10 weeks of cold stratification (8 h: 2 °C & full light; 16h: 2 °C & dark). Subsequently, the incubator was set on 4 weeks of the intermediate regime and finally on 14h of 20 °C & full light and 10h of 10 °C & dark. Daytime always had full light (240 mol m^{-2} s^{-1}) and night always complete darkness. In addition, to control for the potential germination capacity of the seeds, three Petri dishes lined with moist filter paper were filled with 50 randomly selected seeds of each species and exposed to the same climate conditions as the pots.

The final germination percentage was determined after 15 weeks under the final temperature and light regime by counting the number of germinated seeds and weighting the dry aboveground biomass in each replicate pot. We only show the results for *L. luzuloides* (control germination of the seeds: 66 %) and *P. elatior* (25 %). The germination in the Petri dishes of *A. nemorosa* and *G. odoratum* was too low to analyse the data (respectively no germination and less than 5 % germination).

5.2.3 Data analysis

For each species, we calculated a vegetative and regenerative performance index and tested the effects of the experimental treatments. An overview of the measured vegetative and regenerative traits for each species is shown in Table 5.1. When more than one vegetative or regenerative trait was measured, a principal components analysis (PCA) on the traits was performed and the extracted first axis was used as proxy of performance (Baeten *et al.*, 2010b). When appropriate, the trait values were square root or log-transformed to increase the proportion of variance extracted. For each species, the first axes extracted at least 67 % of the variation in either vegetative or regenerative traits. Each trait value showed a positive correlation with its associated PCA axis (i.e., there were no trade-offs among traits), the Pearson correlation coefficient between all traits and the first PCA axis was always higher than 0.73 (all significant at $P < 0.0001$) for the vegetative traits and higher than 0.91 (significant at $P < 0.0001$) for the regenerative traits. For the germination experiment, the germination of the seeds and the aboveground biomass of the seedlings was highly correlated (cor > 0.68; $P < 0.0001$). Therefore, we calculated a germination index, analogous to the vegetative and regenerative performance, using the first axis of a PCA on the number of seeds germinated and the total aboveground biomass of seedlings. The mean Pearson correlation coefficient between a trait and the first PCA axis was always higher than 0.90 (all significant at $P < 0.0001$). All indices were rescaled to values between 0 (lowest value) and 1 (highest value).

Table 5.1. Overview characteristics and the traits measured for each of the five study species. Soil acidity based on Ellenberg and Leuschner (2010) and flowering period on De Langhe *et al*. (1995). Changes in abundances after conversion from deciduous stands to spruce plantations based on the results of Verstraeten *et al*. (2013a).

Species	***Anemone nemorosa***	***Convallaria majalis***	***Galium odoratum***	***Luzula luzuloides***	***Primula elatior***
Species Characteristics					
Soil acidity	Indifferent	Indifferent	Indicator of weakly acid to weakly basic conditions	Acidity indicator	Indicator of moderate to weakly acid soils
Flowering period	March – May	May – June	May – June	May – June	March – May
Leaf phenology	Vernal	Aestival	Aestival	Evergreen	vernal-aestival
Live form	Geophyte	Geophyte	Hemicryptophyte	Hemicryptophyte	Hemicryptophyte
Change in abundance after conversion	Lower	Lower	Lower	Higher	Lower
Vegetative measurements					
Total number of basal leaves		X			X
Length of longest leaf	X	X	X		X
Total aboveground dry biomass *	X	X	X	X	X
Number of ramets	X				
Plant height				X	
Length longest stolon			X		
Number of stolons			X		
Regenerative measurements					
Proportion of flowering ramets	X				
Number of inflorescences		X		X	
Proportion of flowering stolons			X		
Height of highest inflorescence					X
Number of flowers					X

* The total aboveground biomass was measured as the harvested aboveground biomass dried for 72 h at 70 °C, and weighed (± 0.1 mg).

The vegetative and regenerative performances were tested using hierarchical models: performance ~ *light regime* + *tree species* * *site type* with intercepts varying randomly by plot. Variation in germination was tested using general linear models with following model: germination index ~ *tree species* * *site type*. All statistic analysis where performed with R 2.15.2 and using the *lme* function of the *nlme* library (R Development Core Team 2010).

5.3 Results

5.3.1 Vegetative performance

The light regime was an important factor in determining the vegetative plant performance for all species except *P. elatior* (Table 5.2). For *L. luzuloides* and *C. majalis* the light regime was the only significant factor. Light regime and the interaction between tree species and site type were significant for *A. nemorosa*. For *G. odoratum*, also site type was a significant factor to explain the variation in plant performance. The vegetative performance of *A. nemorosa* was lowest for the evergreen light regime and highest for the light deciduous regime. Furthermore, *C. majalis*, *G. odoratum* and *L. luzuloides* showed the highest plant performance for the light deciduous light regime, but the lowest performance for these species was for the dark deciduous regime. The vegetative performance of *G. odoratum* depended also on the site type, this species had a higher performance in soil of the less acidic sites. The variation in vegetative performance of *P. elatior* was mainly determined by site type as only significant factor, with higher performance on the less acidic sites (Table 5.2, Figure 5.1).

5.3.2 Regenerative performance

For *P. elatior* and *G. odoratum*, the regenerative performance was higher in pots with soil from the less acidic sites. The regenerative performance of *L. luzuloides* was very low for plants of the dark deciduous light regime. For *G. odoratum* and *A. nemorosa*, the regenerative performance was lower in both the dark deciduous and evergreen light regime compared with the light deciduous regime. The regenerative performance of *C. majalis* was independent of the factors in the model, but these measurements of performance were done after one growing year.

Table 5.2. Vegetative and regenerative performance of the study species. Hierarchical model: performance ~ LIGHT REGIME + TREE SPECIES * SITE with intercepts varying random by plot. Reference level for each treatment: LIGHT REGIME: deciduous light, TREE SPECIES: deciduous, SITE: most acidic site. Other treatments levels are tested against the reference level. Significance: (*) $P \leq 0.1$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

	Anemone nemorosa			*Convallaria majalis*			*Galium odoratum*			*Luzula luzuloides*			*Priumula elatior*		
	parameter estimate	t-value		parameter estimate	t-value		parameter estimate	t-value		parameter estimate	t-value		parameter estimate	t-value	
vegetative performance															
Intercept	0.5	7.94		0.54	6.75		0.69	11.46		0.7	16.36		0.5	5.55	
Light regime - deciduous dark	-0.13	-2.01	(*)	-0.31	-3.54	*	-0.43	-7.42	***	-0.48	-9.07	***	-0.06	-0.72	
Light regime – evergreen	-0.28	-4.4	***	-0.12	-1.46		-0.25	-4.03	**	-0.14	-2.79	*	-0.07	-0.6	
Tree species – spruce	0.15	1.87	(*)	0.16	1.67		-0.04	-0.58		-0.08	-1.96	(*)	0.07	0.89	
Site type – less acidic site	0.05	0.66		0.03	0.25		0.24	3.04	**	-0.03	-0.81		0.25	3.01	**
Tree species * site type	-0.22	-2.04	*	-0.02	-0.15		-0.15	-1.47		0.05	0.87		-0.22	-1.94	(*)
regenerative performance															
Intercept	0.27	1.82		0.58	6.1		3.4	9.25		9.55	5.19		0.59	6.47	
Light regime - deciduous dark	-0.37	-2.35	*	0.13	1.25		-2.96	-8.32	**	-8.92	-3.83	**	-0.09	-1.12	
Light regime – evergreen	-0.31	-2.04	*	-0.18	-1.91		-2.83	-7.63	**	1.53	0.67		-0.07	-0.62	
Tree species – spruce	0.21	1.12		0.03	0.29		-0.07	-0.17		-2.18	-1.42		0.07	0.76	
Site type – less acidic site	0.12	0.71		0.05	0.38		1.26	2.6	*	1.47	0.96		0.2	2.12	*
Tree species * site type	0.01	0.04		0.01	0.08		-1.07	-1.72	(*)	-1.01	-0.46		-0.2	-1.49	

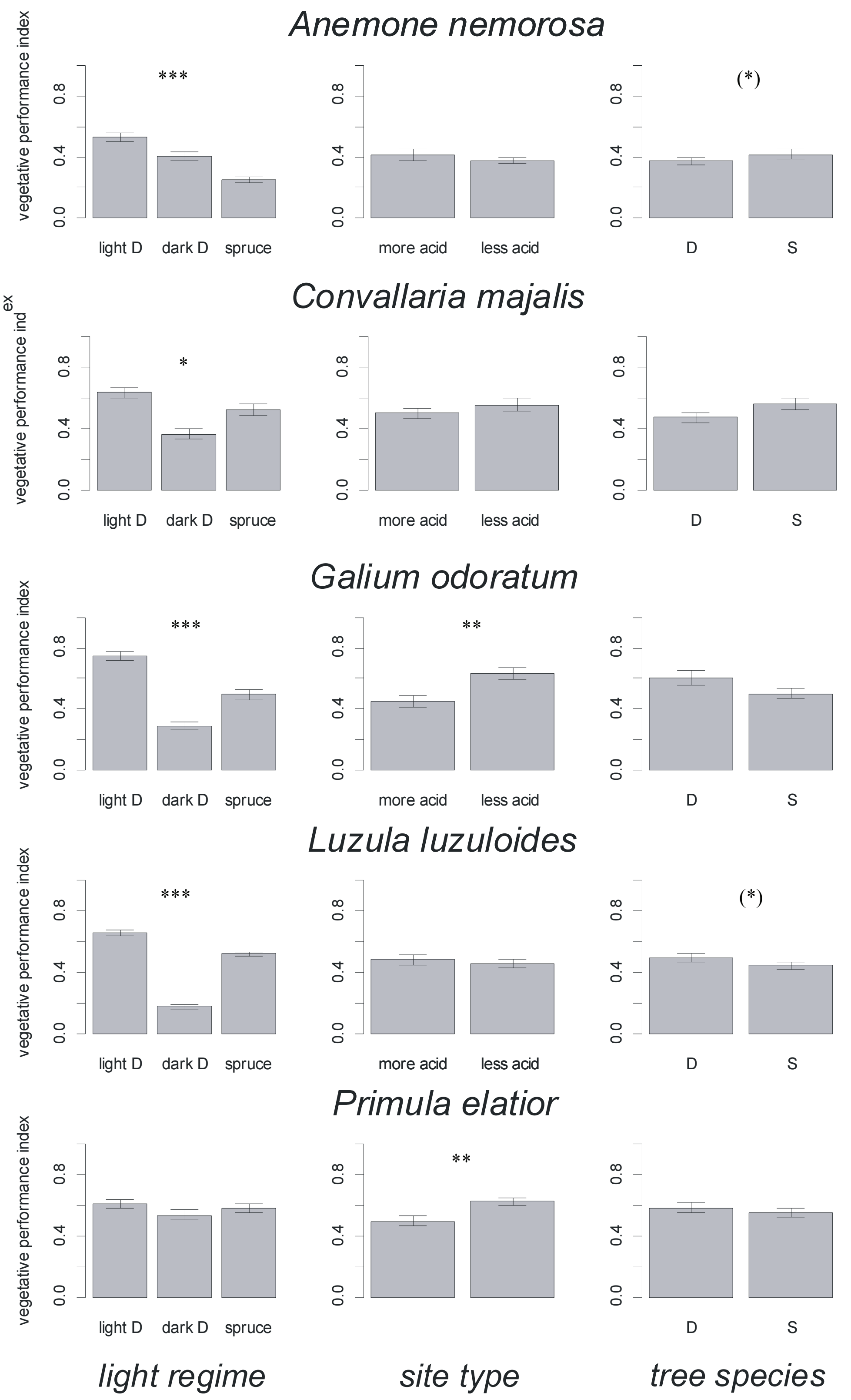


Figure 5.1. Vegetative performance (mean ± SE) of the five studied species for the factors light regime (light D = light deciduous; dark D = dark deciduous, site type and tree species (D = deciduous; S = spruce). Significance: (*) $P \leq 0.1$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

5.3.3 Germination experiment

The germination index of *P. elatior* and *L. luzuloides* was higher in pots with soil of the deciduous stand compared with soil of spruce stands (Figure 5.2). The difference between the sites was slightly significantly higher on the less acidic sites for *L. luzuloides*. For *P. elatior*, the germination index was clearly higher on the less acidic sites.

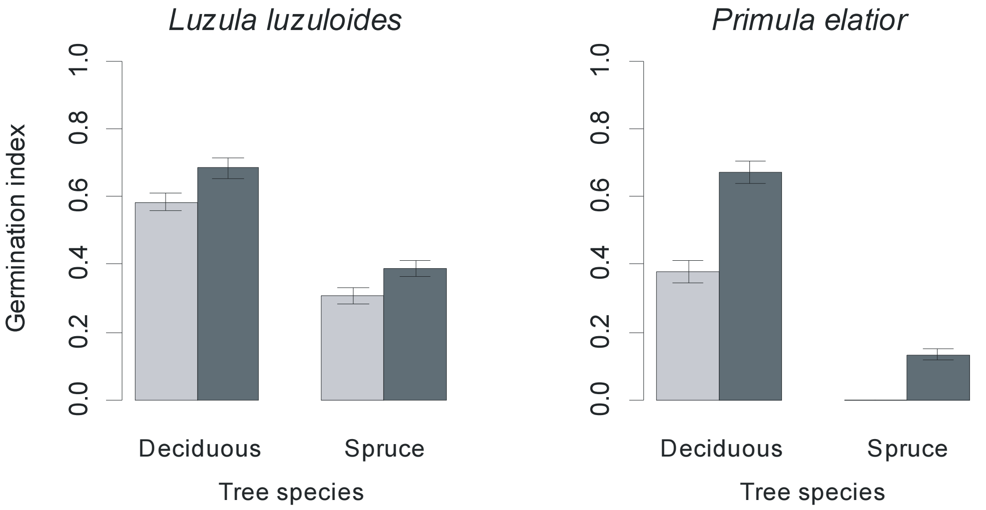


Figure 5.2. Germination index (mean ± SE) of *Luzula luzuloides* and *Primula elatior*. Anova results: *L. luzuloides*, factor *site type* F=5.07*, factor *tree species* F=41.38***, interaction NS. *P. elatior*, factor *site type* F=25.94***, factor *tree species* F=126.96***, interaction 4.09*. Light grey: acidic sites. Dark grey: moderately acidic sites.

5.4 Discussion

Forest conversion using contrasting tree species has been shown to have large impacts on forest ecosystem functioning. The overstorey composition also significantly influences the understorey composition (Palik and Engstrom, 1999; Barbier *et al.*, 2008) and therefore changes in the understorey layer are expected after conversion. Tree species influence the understorey vegetation through modification of resource availability and quality (light, water and nutrients) and other effects such as the physical impact on the soil by roots and physical effects of litter (Barbier *et al.*, 2008). Our observational study

(Verstraeten *et al.*, 2013a) confirmed that the conversion from mixed deciduous to spruce stands changed the understorey composition, with changes in abiotic soil characteristics and light regime as important drivers. Here, we experimentally assessed the effects of these variables on five understorey plant species with different sensitivity for acidity and light regime. Below, we discuss the effects on the performance and germination, and compare the experimental results with the results of the observational study.

The light regime appeared as an important factor for vegetative and regenerative performance for four of the five studied species, but the species showed a different response to this factor. *A. nemorosa* was the only species with a significant lower performance for the "evergreen" light regime compared with the two deciduous light regimes. *A. nemorosa* is a vernal species: the light availability on the forest floor at the end of the winter and early spring is important for this species. Peterken (1993) stated that planting coniferous species largely eliminated spring-growing understorey species; lower light availability in winter and spring lowered the performance of these species. The lower light availability in winter of the evergreen regime also had an effect on the vegetative performance of *G. odoratum* and *L. luzuloides*. For aestival (*C. majalis*, *G. odoratum*) and evergreen species (*L. luzuloides*), the light availability after tree canopy closure of the deciduous trees was more important. The performance of these species was lowest for "dark deciduous" regime. Baeten *et al*. (2010b) also experimentally studied the effect of summer light levels on four forest species; the light level in summer was also important for the performance of the two summer-flowering forest species and had no effect on the performance of the two spring-flowering species (also *A. nemorosa* and *P. elatior*). We did not study the effect of a 'summer dark and evergreen light regime' that might occur in less intensively managed coniferous plantations; so the focus in the present study was on regularly managed plantations. For all the studied species, both 'evergreen' and 'summer dark' light regimes together are expected to lower their vegetative performance and this expectation could be studied analogous to our experiment.

In this experiment, soil was taken in sites with rather more acidic soils and sites with less acidic soils. Only the acid sensitive species (*P. elatior* and *G. odoratum*) responded to this treatment with higher vegetative and regenerative performance in pots filled with soil of the less acidic sites. Similarly, the potexperiment of Falkengren-Grerup and Tyler (1993) found a response of all species to pH, but the response differed between species: species with high frequencies on highly acidic soils had a lower performance on the very acidic situation (soil solution pH-H_20 interval: 3.3 – 5.5). Our sites were less acid than the most acidic situation in their study. Therefore, it is possible that our most acidic sites did not have a pH that was low enough to find a response for the indifferent and acid-tolerant species. Falkengren- Grerup and Tyler (1993) also treated an acidic soil with carbonates to get different pH levels, which is not the same as soil collected along a real pH-gradient as in our study.

The species distribution of the studied species in deciduous stands is in accordance with the experimental results. For example, *G. odoratum* and *P. elatior* had higher performance in the experiment in pots with soils of the less acidic sites and were more abundant in the field on less acidic soils. The acid-tolerant species did not show a lower performance on the richer sites, which is in accordance with other experimental studies (Brunet and Neymark, 1992; Falkengren-Grerup and Tyler, 1993; Thomaes *et al.*, 2011). The results of the experiment give more information about the fundamental niche of the species, i.e. in the absence of competition, and the realised niche under real forest conditions is expected to be smaller. The higher competition on the less acidic sites could explain the lower abundances of the acid-tolerant species, rather than their preference for the most acidic sites. Temporal changes in understorey diversity and composition in deciduous stands have often been linked to acidification and lower management intensity and the concomitant lower light transmittance (Baeten *et al.*, 2009a; Van Calster *et al.*, 2007; 2009a). The lower light availability in summer will probably be the most important factor that is lowering the performance of several species. For the more moderately acidic species, also acidification will be a driver of change.

However, the performance of adult plants in the experiment was clearly not sufficient to explain the species' distributions in spruce stands. Four of the five species were less abundant in the spruce plots (Table 5.1) while the performance of these plants was not depending on the tree species in the stand where the soil was sampled and the evergreen light regime was for some species even better than the dark deciduous regime common in the study area. The differences will probably be the result of lower germination and establishment of forest herbs in the spruce stand. Germination was influenced by the tree species where the soil was sampled, as was also shown in other studies (Brunet and

Neymark, 1992; Thomaes *et al.*, 2011). The altered abiotic soil conditions after conversion could limit the germination and establishment in the spruce stands. Dispersal limitation could also be a bottleneck for several forest plants after conversion. In case of direct conversion by plantation, the understorey community may be heavily damaged during conversion or may have difficulties to survive the period with very high canopy closure in young stands. Many ancient forest species are not found in the persistent seed bank (Bossuyt and Hermy, 2001; Verheyen *et al.*, 2003a), although recent literature attributes this to a too low sampling intensity (Plue *et al.*, 2012). Ancient forest species also have slow colonisation rates that could limit the recolonisation of these species into the converted stands (Verheyen *et al.*, 2003b; De Frenne *et al.*, 2011). Studies in post-agricultural forests showed that several forest species have limited dispersal capacities (e.g. Matlack, 1994; Bossuyt *et al.*, 1999; Verheyen and Hermy, 2001a, b) whereby also a slow recolonisation of the converted stands could be expected. The higher mass of the forest floor could maybe also have an effect on the performance (Baeten *et al.*2009b), but this factor was not incorporated in this study. For example, *P. elatior* was only found in deciduous stands in our study area, but the experimental vegetative and regenerative performance did not differ between deciduous and spruce tree species and the light regime did not affect the performance of this species. *P. elatior* has no long living seed bank and the low colonising capacity of *P. elatior* (Verheyen *et al.*2003c) will limit the colonisation of this species into the spruce stands. In addition, there was only a limited germination and establishment in spruce soil in this experiment. The germination of *L. luzuloides* was also lower in spruce stands. Nevertheless, this species is an indicator of spruce stands. *L. luzuloides* is the least dispersal-limited of our study species (fastest coloniser according to Verheyen *et al*. (2003a). Furthermore, *L. luzuloides* had a higher performance in stands with higher light transmittance in the experiment. The observed abundance of this species was higher because it takes advantage of the higher light availability in summer under spruce. It is difficult to relate a single variable to the lower germination of both species in soil sampled in spruce stands. The differences in abiotic soil conditions will contribute to the variation in germination index. Several studies showed that the germination and establishment of forest herbs decreased at lower soil pH-values (Brunet and Neymark, 1992; Falkengren-Grerup and Tyler, 1993; Thomaes *et al.*, 2011). Therefore, the germination was expected to be lower in pots with soil of spruce stands, but pH alone was definitely not enough to explain the large differences in germination between deciduous and spruce stands. The spruce stands from less acidic sites had a comparable pH with the deciduous stands from locations on most acidic sites, but the germination was significantly lower. Other factors will probably be important here: also mycorrhiza infection rates, root exudates and soil fauna community change after conversion. Changes of these and other variables and their effects on the germination of plant species are less known. Further research focussing on the factors causing the lower germination in spruce soil could be worthwhile. Also the direct effects of the thickness and structural differences of the forest floor could influence the germination and performance of these species (Barbier *et al.*, 2008), but they were not studied here. Other studies showed that a thick forest floor litter layer has not always detrimental effects on the performance of forest species (Sydes and Grime, 1981; Eriksson, 1995; Baeten *et al.*, 2009b), but it was only studied for deciduous species.

To conclude, species-specific responses of understorey plant species to abiotic changes after forest conversion are important in determining the understorey community composition and diversity. For the adult plants of this study, the differences in performance between soil sampled in deciduous stands and spruce plantations were not significant. Light transmittance, on the other hand, both the quantity as well as the periodicity was clearly an important factor both for the vegetative and regenerative performance of understorey species. The lower germination and establishment in soil sampled in spruce stand will be also an important cause of the different species composition after conversion.

Experimental conversion study, the two plots at the right sight are changed soil monoliths. Gaume, 2011

6

Conversion of deciduous stands to spruce plantations and back: experimental evaluation of effects on soil, forest floor and earthworms

Abstract

Changes in tree species composition from native deciduous forest to spruce plantations often result in soil acidification, a build-up of the forest floor, retarded nutrient cycling and changes in the understorey composition. Reconverting these secondary non-native coniferous plantations into more natural deciduous forests became a main aim of sustainable forestry, but the restoration possibilities of these acidified ecosystems are unclear. Here we assess the effects of two opposite land cover changes (a conversion pathway from deciduous to spruce, and a restoration pathway from spruce to deciduous) on the forest floor, soil acidification and earthworm community. This experiment was carried out in the Gaume, an area with a mild temperate climate in southern Belgium where spruce plantations were established 35–50 years ago in a matrix of deciduous woodlands. We changed soil monoliths from the deciduous forest to the spruce plantations (conversion pathway) and vice-versa for the restoration pathway and applied two additional treatments: +/- liming and +/- earthworm access. Two and a half year after transferring the deciduous soil in the spruce stand we already found the forest floor mass to be significantly higher and the earthworm biomass to be significantly lower under spruce cover. Soil pH and exchangeable calcium and aluminium concentrations were not influenced. In the restoration pathway, the forest floor mass was significantly lowered, soil pH and exchangeable calcium concentrations were significantly increased and exchangeable aluminium concentrations significantly decreased. The earthworm biomass under deciduous tree cover was twice as large as under spruce tree cover. In both the conversion and the restoration pathway, lime addition caused an increase of the pH up to 2 pH-units while the earthworm access treatment had no effect. This experiment confirms that conversion to spruce plantations has rapid significant negative effects on the forest floor and the earthworm community. Our short-term experiment further demonstrates

that changing the tree species cover back to deciduous tree species initiates a trajectory towards ecosystem restoration (especially in combination with lime addition).

6.1 Introduction

During the 19th and 20th century, large areas of native deciduous forests in Western and Central Europe were converted to coniferous plantations (Klimo *et al.*, 2000; Spiecker *et al.*, 2004). Conversions were mainly motivated by an economic interest; coniferous species grow faster and produce highly demanded industrial wood. A frequently used species for conversion was Norway spruce (*Picea abies*, further referred to as "spruce"). The composition of the forest overstorey has an impact on the chemical, physical and biological characteristics of the soil (Augusto *et al.*, 2002). So, changes in tree species composition can have strong impact on ecosystem functioning. Spruce is generally considered to be a tree species with strong ecological effects due to its recalcitrant leaf litter and its large and permanent leaf area, leading to higher capturing of atmospheric pollutants (Ranger and Nys, 1994; Augusto *et al.*, 2002; De Schrijver *et al.*, 2007). Conversion of deciduous forests into spruce plantations often resulted in soil acidification, an accumulation of organic matter in the forest floor, retarded nutrient cycling and changes in the understorey composition (e.g. Binkley and Valentine, 1991; Emmer *et al.*, 1998; Ewald, 2000; Máliš *et al.*, 2012; Verstraeten *et al.*, 2013a). Spruce plantations are also vulnerable for abiotic and biotic damages, for example higher risk for windfall and outbreaks of spruce bark beetle (Spiecker, 2000; Solberg, 2004). The natural range of spruce in Europe are the boreal forests and the mountains (Schmidt-Vogt, 1977), but spruce is also planted far outside its natural range. In times of rapid climate change, cultivation of species close to and beyond their distributional limits involves serious risks (Bradshaw *et al.*, 2000). Therefore, reconverting these secondary non-native coniferous plantations into more natural deciduous forests has become an important topic for forest management (Mosandl and Küssner, 1999; Spiecker *et al.*, 2004). Through this reconversion, restoration of the impoverished and acidified soils is strived for. Several studies investigated the effects of reconverting conifer plantations to deciduous or mixed stands (e.g. Zerbe, 2002; Heinrichs and Schmidt, 2009; Felton *et al.*, 2010). Changing the tree species composition from spruce to deciduous tree species will increase the leaf litter quality and accelerate the decomposition of the forest floor, resulting in a lower acidifying input in the soil. Restoration could be strengthened and accelerated by liming to neutralize accumulated soil acidity. Lime addition reduces the H^+ ion concentrations and adds calcium ions to the soil exchange complex and soil solution which result in a decrease of the soil acidity and an improvement of the cation exchange capacity and base saturation. Liming is also stimulating the soil biota (e.g. Lang and Beese, 1985; Theenhaus and Schaefer, 1995; Deleporte and Tillier, 1999), which typically results in a faster decomposition of the forest floor. Much less studied is the impact of earthworm introduction on the restoration process. However, earthworm introduction (mainly anecic and endogeic species) can improve the physical and chemical soil conditions due to their bioturbation activity, which affects the forest floor and nutrient turnover (Huhta, 1979; Brun *et al.*, 1987; Deleporte and Tillier, 1999).

Integrated restoration with a combination of abiotic (neutralisation of the acidification by liming) and biotic restoration measures (e.g. conversion to tree species with fast decomposing litter and increase of the earthworm community) can possibly increase the chances for successful restoration (Muys *et al.*, 2003). Liming will decrease soil acidity, improving the soil conditions for soil biota as endogeic earthworm species, while a stimulated earthworm community can in turn enhance the effects of liming. Especially endogeic and anecic earthworms species have a beneficial effect on the dispersal of lime by burrowing activities (Baker *et al.*, 1999; Chan, 2003). Here, we tested and compared the effects of two opposite land cover changes (a conversion pathway from deciduous to spruce, and a restoration pathway from spruce to deciduous) on soil acidity and characteristics of the forest floor and of the earthworm community. We wanted to know whether the effects of conversion and restoration on these variables are already visible after a short time period (two and a half year) and whether the process is reversible; i.e. whether the restoration process follows the same pathway as conversion but in the opposite direction. We tested these questions in an experiment where we simulated conversion and restoration by exchanging soil monoliths between adjacent deciduous and spruce stands. We also studied whether lime addition and/or earthworms are stimulating or slowing down the conversion or the restoration paths.

6.2 Materials and methods

6.2.1 Study site

The study area was a part of a large forest complex in the south of Belgium (Gaume region, described in chapter 1.3.1).

6.2.2 Study design

To study the effect of conversion and restoration on the soil, the forest floor characteristics and the earthworm community, we selected six pairs of adjacent deciduous and spruce stands distributed along a natural gradient of soil buffering capacity. This site gradient, described in Verstraeten *et al.* (2013a), was confirmed by soil characteristics (pH, exchangeable calcium and aluminium concentrations and clay content measured in the broadleaved matrix). In November and December 2009, we established in each deciduous and spruce stand two square experimental plots of 1.6 m x 1.6 m subdivided in four subplots of 0.8 m x 0.8 m. The centre of the plots was situated about 30 m from the border of the adjacent stand (Figure 6.1) and thus about 60 m from the plots in the adjacent stand. This distance of 30 m (about one tree height) was chosen as an optimum to minimize site differences, as well as mutual influences between the stands. The distance between the two plots in each stand was about two meter (Figure 6.1). We simulated changes in tree species composition by exchanging soil monoliths from the deciduous stand to the spruce stand to simulate the conversion pathway, and from the spruce stand to the deciduous stand to simulate the restoration pathway. The soil exchange was done for two randomly selected subplots of each plot, while the other two subplots remained in their original position. In the transported subplots, we excavated soil monoliths in four undisturbed squares of about 0.4 m * 0.4 m and 15 cm deep. These monoliths were displaced in a subplot of the adjacent stand (Figure 6.1). The transport and the installation of the soil monoliths were done with great care to minimize structural changes in the soil.

We further performed two additional treatments: a lime addition and an earthworm treatment. The liming treatment was done by adding twice (May and December 2010) 180 g dolomite (equivalent to 2812 kg ha^{-1}, 60% calcium carbonate, 30% magnesium carbonate, 2-10 mm granules) on the top of the forest floor (cf. Hultberg *et al.*, 1995; Dulière *et al.*, 2000). The lime was added in two of the four subplots of each plot. Introduction of earthworms can only give good results in case appropriate abiotic conditions are present during and after introduction. We here opted not to introduce earthworms, but to allow or obstruct earthworm migration between the subplots and the surrounding forest floor and soil. In case of no obstruction, we expected the earthworm community to expand as a consequence of immigration in case of improved abiotic conditions. Therefore, we could interpret the obstruction of migration as ‘no introduction’ and allowing migration as ‘natural introduction in case of good abiotic circumstances’. The earthworm obstruction was done by placing wooden panels around a subplot (15 cm deep, 10 cm above the ground) to obstruct earthworms and other soil biota from entering the subplot. Around the other subplots, we placed similar wooden panels with big holes to minimize any difference in e.g. microclimate. In each plot, earthworms were obstructed from migration in two subplots and allowed to migrate in the two other subplots.

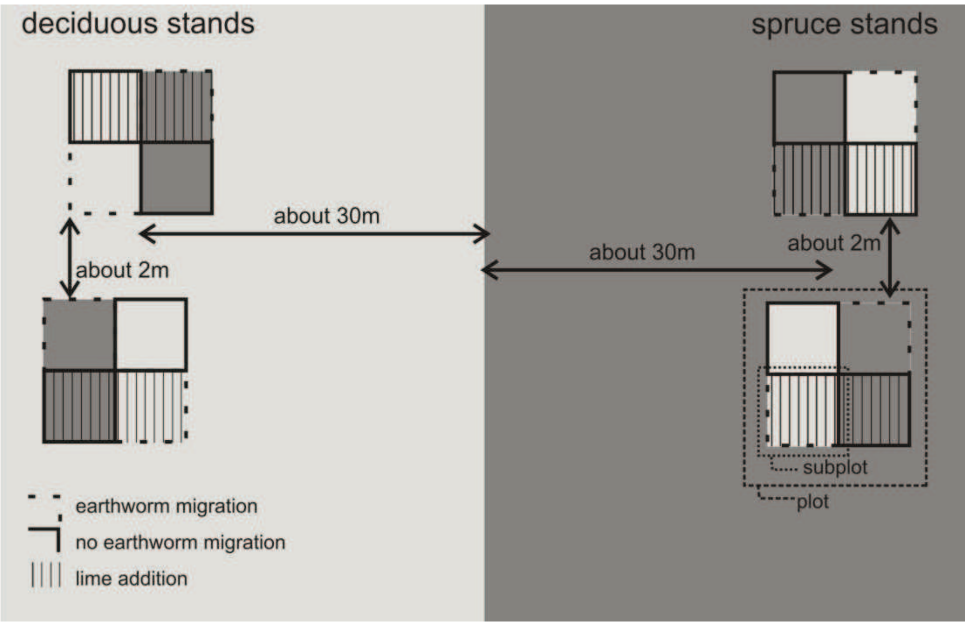


Figure 6.1. Schematic overview of the experimental design. Light grey shows soil originating from the deciduous stand and dark grey shows soil origination from spruce stands. Plot size is 1.6 m x 1.6 m and subplot size is 0.8 m x 0.8 m. Earthworm migration was obstructed with wooden panels. For the conversion pathway we compared deciduous soil in the deciduous stands with soil originating from the deciduous stands in the spruce stands (light grey subplots). For the restoration pathway we compared spruce soil in the spruce stands with soil originating from the spruce stands in the deciduous stands (dark grey subplots). This design was repeated on six different sites.

In each stand we applied three treatments (soil origin, lime addition and earthworm migration) with each two levels. This makes eight combinations and thus eight subplots in each stand. We spread the subplots over two square plots so that each subplot has two sides adjacent to the surrounding forest stand and the two other sides adjacent to another subplot.

6.2.3 Data collection

We measured response variables in three categories: soil variables concerning acidification, the forest floor and the earthworm community. Soil variables were measured at the beginning of the experiment in each subplot. At the end of the experiment (May 2012), nearly two and a half years after establishment of the experiment, we measured all the response variables. We first removed the vegetation carefully. Next, we took the forest floor samples for measuring the forest floor biomass and the earthworms of the forest floor, then the soil samples were taken and finally we performed the earthworm sampling in the soil.

Topsoil samples (0-5 cm of the mineral soil) were analysed for variables in relation to soil acidity. In each subplot, we randomly collected three mineral soil samples after removal of the forest floor by using a plastic tube of diameter 3.3 cm. At the beginning of the experiment, we took the soil samples at the border of the subplots to avoid disturbances. The three soil samples of each subplots were pooled into a composite soil sample. The soil samples were dried at 40°C until constant weight and analysed for pH (extraction in 1 M KCl, 1:5 suspension, ion-specific electrode) and exchangeable calcium (Ca^{2+}) and aluminium (Al^{3+}) concentrations (extraction in 0.1 M $BaCl_2$, 1:20 suspension, Dutch standard method NEN 5738; flame atomic absorption spectrophotometry, SpectrAA-220, Varian).

Forest floor samples were collected in one square of 20 cm x 20 cm in each subplot. The two samples of the forest floor from the earthworm sampling (see further) were also used as measurement of the total forest floor mass. These samples were dried at 70°C until constant weight and weighted.

Earthworms were sampled in tree steps. In a first step, the earthworms present in the forest floor were collected by placing two smaller squares of 0.2 m x 0.2 m on the forest floor in the middle of the big square and collected the forest floor in plastic bags.

Earthworms present in the forest floor were collected by hand-sorting the plastic bags. The forest floor outside the small squares was removed. In a second step, earthworms were extracted from the soil by using 30 litres of mustard suspension per plot (6 g mustard powder per litre) in a bigger square (0.5 x 0.5 m) (Valckx *et al.*, 2011). The frequency and velocity of pouring depended on the percolation rate of the solution. The earthworms were collected directly after emerging and were stored on ethanol. In a third and last step, the mustard affected area was controlled for earthworms by handsorting a soil monolith (0.2 m x 0.2 m and 0.2 m depth). All earthworms were first stored in ethanol and after a few hours transferred to a 5 % formalin solution for fixation. After 3 days, they were again transferred to 95 % ethanol solution for further preservation and identification. In the laboratory, earthworms were determined to species level following the nomenclature of Sims and Gerard (1999), their developmental stage was recorded ((sub)adult vs. juvenile) and weighted with gut contents. Earthworm mass was expressed as g m^{-2} formalin-preserved weight. In the few cases that determination to species level was not possible, earthworms were identified at genus or ecological group level and were assigned pro rata to species level (cf. Valckx *et al.*, 2006).

6.2.4 Data analysis

We both looked into the effects of the conversion pathway (conversion of deciduous forests to spruce plantations, using only data of subplots with soil origination from deciduous stands) and of the restoration pathway (reconversion of spruce plantations to deciduous forests, using only data of subplots with soil originating from spruce stands). We used linear mixed effect models and a model selection procedure according to Zuur (2009) to test the effects of the tree species cover, lime addition and earthworm migration on soil characteristics related to acidity, forest floor weight and the earthworm community. An optimal model was selected by comparing models with different random structure but equal full factorial fixed effects. A model with the intercepts varying randomly by site had the lowest AIC value and was selected. Then, we optimized the fixed effects structure and removed the interactions and explanatory variables successively based on likelihood ratio tests, i.e., if the fit of model is not significantly reduced when an effect is removed. Finally we obtained the optimal model for each response variable when no interaction or explanatory variables could be dropped out the model without reducing the model fit. A model was selected for each response variable in both the conversion and restoration pathway. All statistical analyses were performed with

R 2.15.2 and using the *lme* function of the *lmne* library (R Development Core Team 2010).

6.3 Results

6.3.1 The conversion pathway: from deciduous to spruce forest

For studying the conversion of deciduous forest to spruce forest, we compared the subplots with deciduous soil in the deciduous stand (tree species cover: deciduous) with the subplots of deciduous soil in the spruce stands (tree species cover: spruce). Two and a half year after transferring the deciduous soil monoliths in the spruce stand, the forest floor mass was significantly higher under the spruce tree cover compared to deciduous tree cover (Table 6.1, Figure 6.3). Also the earthworm biomass was significantly influenced by the tree species cover, with significantly lower earthworm biomasses under spruce tree cover compared to deciduous tree cover (Table 6.1, Figure 6.4). Changing tree species cover to spruce did not have an influence (yet) on the soil variables concerning acidification at the end of the experiment.

Lime addition caused a significant higher soil pH and significantly higher exchangeable calcium concentrations (Table 6.1) in both stand types. Exchangeable aluminium concentrations were significantly lower after addition of lime (Table 6.1). The median pH increased with nearly 2 pH-units in the limed plots of both stands (Figure 6.2). The median values of earthworm biomass in limed plots were about double of the median values in plots without lime addition, but this was not a significant effect. The interaction between the earthworm treatment and liming was significant; the earthworm biomass was equal in the plots without liming, but was slightly higher in the limed plots with earthworm obstruction compared to the limed plots without earthworm obstruction.

Table 6.3. Results of the linear mixed effect models for the conversion (soil from deciduous stands) and reconversion (soil from spruce stands) case. Results (t-values) and p-value only are shown for explanatory variables and interactions present in the optimal model. The interaction between lime, tree species cover and earthworm obstruction was not present in an optimal model for any response variables. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Treatment	Intercept	Lime	Tree species cover	Earthworm obstruction	Lime : tree species cover	Lime:earthworm obstruction	Tree species cover:earthworm obstruction
Conversion pathway							
Soil variables (0 - 5 cm layer)							
pH-KCl	21.81 ***	12.27 ***	-	-	-	-	-
Calcium concentration (log)	24.05 ***	8.17 ***	-	-	-	-	-
Aluminium concentration	0.43 NS	-5.81 ***	-	-	-	-	-
Forest floor							
Forest floor mass	5.46 ***	-	4.47 ***	-	-	-	-
Earthworm community							
Earthworm biomass	6.83 ***	-0.15 NS	-2.87 **	0.35 NS	-	-2.42 *	-
Restoration pathway							
Soil variables (0 - 5 cm layer)							
pH-KCl	31.88 ***	6.33 ***	4.44 ***	-	2.84 **	-	-
Calcium concentration (log)	47.7 ***	9.04 ***	3.57 ***	-	-	-	-
Aluminium concentration	1.64 NS	-6.85 ***	-3.2 **	-	-	-	-
Forest floor							
Forest floor mass	10.59 ***	0.85 NS	-4.53 ***	-	-2.33 *	-	-
Earthworm community							
Earthworm biomass	5.26 ***	-	2.67 *	-	-	-	-

6.3.2 The restoration pathway: from spruce to deciduous forest

To study the restoration pathway, i.e. the reconversion of spruce forest to deciduous forest, we compared the subplots with spruce soil in the spruce stand (tree species cover: spruce) with the subplots of spruce soil in the deciduous stands (tree species cover: deciduous). Two and a half year after transferring the spruce soil in the deciduous stand, we found the forest floor mass to be significantly lower under the deciduous tree cover (Table 6.1, Figure 6.3). Also the soil pH and the exchangeable calcium and aluminium concentration were significantly influenced by the tree species cover (Table 6.1). Changing the tree cover to deciduous species increased the soil pH and the exchangeable calcium concentrations and lowered the exchangeable aluminium concentrations. The median earthworm biomass under the deciduous tree cover was twice as large as the median earthworm biomass under spruce tree cover (Table 6.1, Figure 6.4).

Also liming significantly increased soil pH and exchangeable calcium concentrations, while exchangeable aluminium concentrations significantly decreased (Table 6.1). However, liming did not have a significant effect on the forest floor nor the earthworm community and there was no effect of the earthworm treatment on the reconversion pathway.

The interaction between tree species cover and liming also had a significant effect on soil pH: transferring soil from a spruce stand to a deciduous tree cover in combination with a liming practice caused a stronger increase of the soil pH than when both factors were solely applied. The pH increased in the limed plots in the deciduous stands with about 1.5 pH unit, while in the spruce stand with only about 0.5 pH unit (Figure 6.2).

6.4 Discussion

We tested in a short-term experiment the effects of two opposite pathways of tree species composition change (a conversion pathway from deciduous to spruce and a restoration pathway from spruce to deciduous) on soil acidification, biomass of the forest floor and biomass of the earthworm community. We exchanged soil monoliths to make it possible to study conversions between tree species in a short time period. Only two and a half years after the start of this tree species conversion experiment, we found a significant impact of the tree species cover on forest floor mass and earthworm population, and this both in the conversion and the restoration pathways. In case of the restoration pathway,

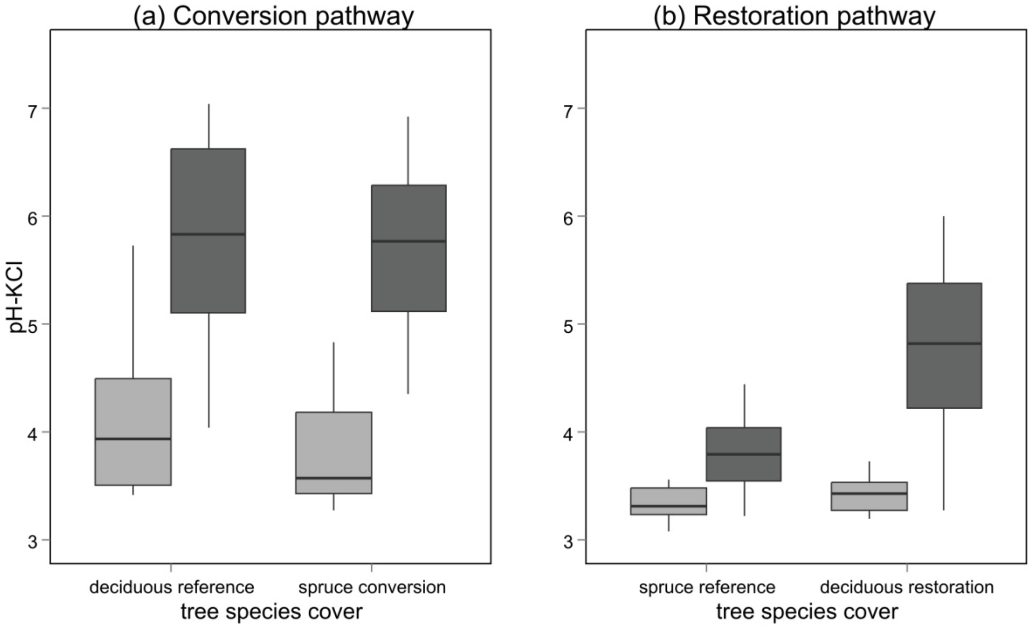


Figure 6.2. Boxplots of the topsoil (0 – 5 cm) pH for (a) the conversion pathway (soil originating from deciduous stands) and (b) the restoration pathway (soil originating from spruce stands). Light grey = no lime addition, dark grey = lime addition.

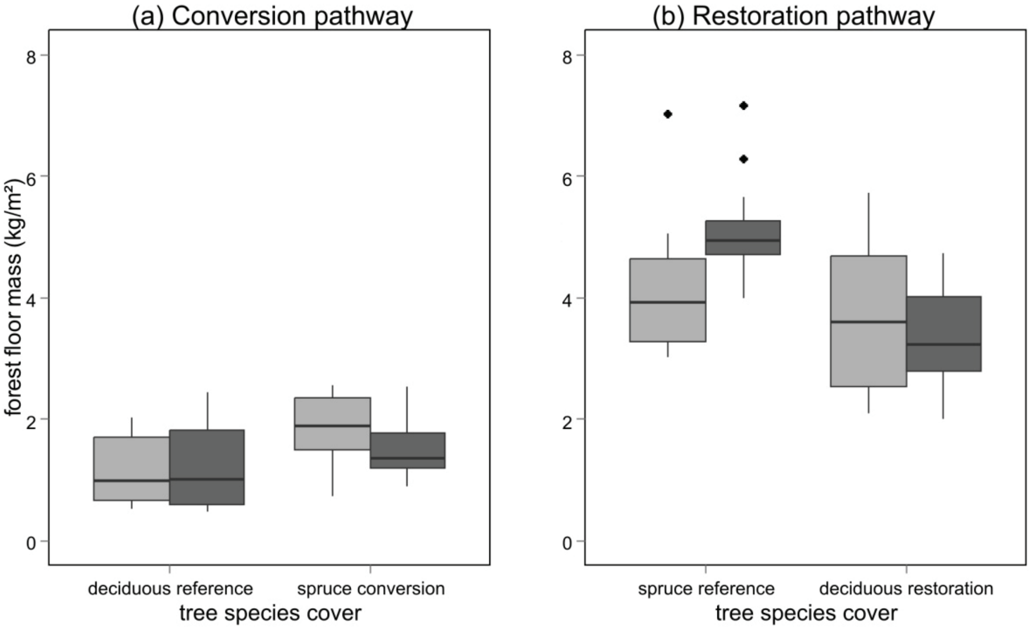


Figure 6.3. Boxplots of the forest floor for (a) the conversion pathway (soil originating from deciduous stands) and (b) the reconversion pathway (soil originating from spruce stands). Light grey = no lime addition, dark grey = lime addition.

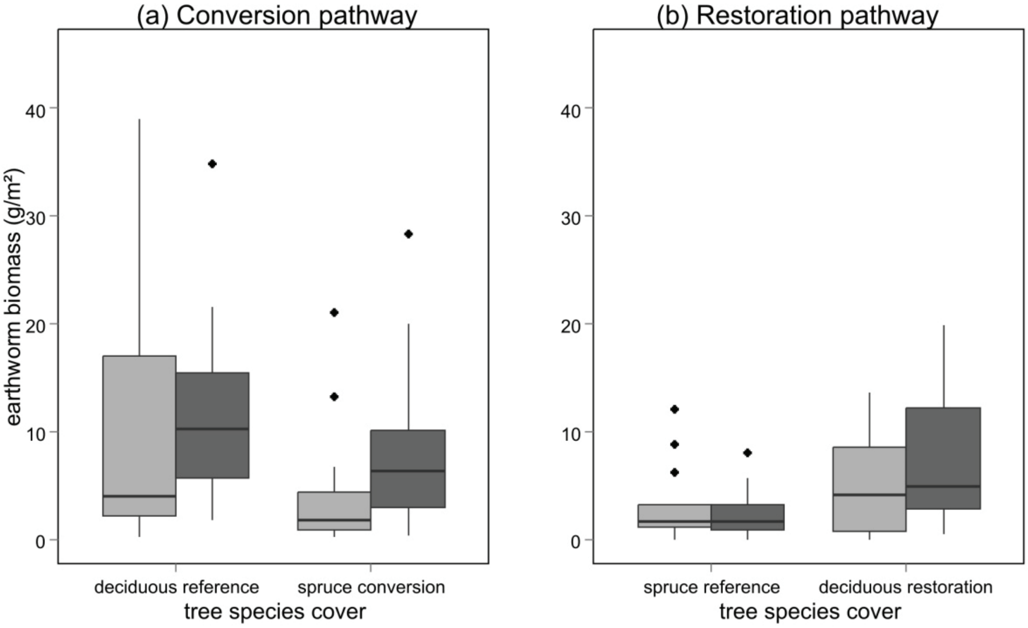


Figure 6.4. Boxplots of the earthworm biomass for (a) the conversion pathway (soil originating from deciduous stands) and (b) the reconversion pathway (soil originating from spruce stands). Light grey = no lime addition, dark grey = lime addition.

where soil of the spruce forest was transferred to a deciduous stand, also soil acidity variables were influenced. We further observed a highly significant impact of a liming practice, both in the conversion and the restoration pathway, while excluding or including the earthworm community did not appear to be of any influence. Here we discuss successively the effect of tree species, lime addition and earthworm obstruction on the soil, the forest floor and the earthworm community in both the conversion and restoration pathways.

6.4.1 Effect of the tree species cover

Based on several observational studies, we expected a strong effect of tree species cover on topsoil characteristics (e.g. Tamm and Hallbäcken, 1986; Augusto *et al.*, 1998; Hagen-Thorn *et al.*, 2004). Spruce is known as an acidifying tree species compared to most deciduous species (Ranger and Nys, 1994; Augusto *et al.*, 1998; Binkley and Giardina, 1998; Reich *et al.*, 2005). As a consequence of its recalcitrant leaf litter properties, spruce litter decomposes slowly and causes a strong build-up of the forest floor (Vesterdal *et al.*, 2008). The lower decomposition rate of spruce needle litter, but also the decreased presence of earthworms (Nordstrøm and Rundgren, 1974) causes forest floor build-up, especially in the H-layer. Tree species cover significantly influenced the forest floor mass in this study. We here hypothesize that this increase in forest floor mass in the conversion pathway is caused by the fact that (i) the low leaf litter quality of spruce and especially the low calcium concentration compared to deciduous species negatively affects the soil biotic community, causing lower decomposition activity (Reich *et al.*, 2005; Hobbie *et al.*, 2006) and (ii) differences in microclimate between the two stand types could change the decomposition velocity of the litter: less light and more constant temperature conditions in winter and often decreased humidity in spruce stands (Augusto *et al.*, 2002) could cause slower litter decomposition. On the other hand, we hypothesize that the forest floor mass in the restoration pathway decreased due to (i) better litter quality of the deciduous trees compared to spruce and (ii) better microclimate for decomposition.

A slow litter decomposition causes soil acidification by the production of organic acids as intermediate decomposition products (de Vries and Breeuwsma, 1985) and by causing a time delay in the cycling of buffering cations (Nilsson *et al.*, 1982). The high LAI and continuous leaf cover of spruce trees causes high interception rates of potentially acidifying pollutants, which gives rise to additional soil acidification (Augusto *et al.*, 2002). In our study, however, conversion to spruce (conversion pathway) did not have a significant effect on the pH and exchangeable calcium and aluminium concentration after two and a half year. Most likely, the time interval of this study was too small to find significant effects. This experiment was also an approach to assess the effects of conversion, but not fully reflecting effective conversion. On the other hand, the soil pH and the exchangeable calcium concentrations increased in the restoration pathway, while exchangeable aluminium concentrations decreased. However, the time interval was probably too small to attribute the significant difference to the absence of the acidifying processes due to slow litter decomposition. Possibly, an accelerated turnover because of changes in microclimate (more light and more extreme temperature conditions in winter and increased humidity) together with the input of faster decomposing deciduous leaf litter and earthworms could have caused a significant difference in this short time period.

The effects of tree species cover on the earthworm community in the conversion and the restoration pathways were clearly visible two and a half year after the start of the experiment: the earthworm biomass was lower under spruce tree cover in both pathways.

This was also found in observational studies to the effects of conversion (Chapter 3) and studies comparing earthworm communities under several different tree species (Reich *et al.*, 2005; Hobbie *et al.*, 2006). The unfavourable litter quality and the lower soil moisture in spruce stands could cause a decrease of the earthworm population.

6.4.2 Effect of liming

Addition of lime in spruce and deciduous stands could have effects on pH and calcium concentrations after less than one year (e.g. Duliere *et al.*, 1999; Misson, 2001). Lime addition caused a dominant effect on the soil variables concerning acidification in all the subplots: where lime was added, the soil variables related to acidification changed in the direction of a less acid situation. Also on the longer time, liming could have a significant influence on the soil variables concerning acidification, especially in topsoil layers (Lundström *et al.*, 2003).

Liming often has a positive effect on the soil biota and earthworm community (e.g. Lang and Beese, 1985; Theenhaus and Schaefer, 1995; Deleporte and Tillier, 1999). We did not find a significant relation with the lime treatment, but Figure 6.4 seems to show a trend with slightly higher earthworm biomass in limed subplots. The variation is large, but the median earthworm biomass tends to be always higher in the limed plots.

Liming could increase the calcium concentrations in the leaf litter (Misson, 2001), making the leaf litter more attractive for endogeic earthworms (Reich *et al.*, 2005). Therefore, the higher calcium concentrations in the leaf litter could enhance the effects of liming on the longer term. This was not the case in our experiment due to the very local addition of lime (only four subplots in each stand), not leading to enriched leaf litter.

6.4.3 Effect of the earthworm treatment

We opted to obstruct and allow earthworm migration by wooden panels in place of introduction. Several studies (e.g. Judas *et al.*, 1997; Muys *et al.*, 2003; Ampoorter *et al.*, 2011) introduced earthworm species to expand the earthworm community and introduce other ecological groups (usually endogeic or anecic species), but often without good survival. Earthworm introduction could work in case of good abiotic conditions for the species. In case of bad abiotic conditions at the time of introduction, the earthworms are usually not found back at the end of the experiment. The earthworm composition and abundances will change and species will migrate according the abiotic conditions and food quality and availability (Muys *et al.*, 2003).

However, the effects of allowing or obstructing earthworm migration on the earthworm community were less obvious. We could not determine significant differences due to earthworm obstruction in any response variable in this study. Possibly, the system of wooden panels to obstruct the earthworm community from migration was not working adequate, there was no migration or the time period was too short. Also, the spatial variation in the earthworm biomass in soils (Edwards and Bohlen, 1996; Nachtergale *et al.*, 2002; Edwards, 2004) makes it also more difficult to find significant differences.

6.4.4 Interaction between tree species cover and liming

The interaction between lime addition and tree species cover was significant for the pH and the forest floor in the restoration pathway. We assume that earthworms and other soil biota play an important role in this interaction because of two possible causes: (i) Possibly, earthworms – especially endogeic species – and other soil biota in the deciduous stand enter the spruce soil monoliths and could enhance the restoration in case of liming. Many earthworm species are acid sensitive and avoid the more acid spruce soil (Chapter 3; Schelfhout, 2010). However, in case of lime addition, the soil pH increased and became more suitable for earthworms and other soil biota so the decomposition rate increased; (ii) Several studies pointed also to the beneficial effect of endogeic and anecic species on the dispersal of lime by burrowing activities (Baker *et al.*, 1999; Chan, 2003). The absence of endogeic earthworm species in spruce stands and the presence in deciduous stands could possibly explain the stronger effects of liming on the soil pH in the deciduous stands in the restoration pathway. In the conversion pathway, the activity of earthworms and other soil biota in the deciduous soil monoliths placed in spruce stands will slowly decrease. This caused no significant changes in pH in this short time period, only a small trend for lower pH under spruce cover is visible in figure 6.2.

6.4.5 Conclusion

Other studies and reviews described many times hysteresis as limiting factor in restoration (Frost *et al.*, 1995; Augustine *et al.*, 1998; Scheffer and Carpenter, 2003): an ecosystem might be easily changed to another state by a disturbance, but removing the disturbance is mostly not enough to return to the original state. Usually it takes more time and another, longer route to reach the original state, if that is even possible at all (Tallis, 1991). The approach of this study tried to assess the effects of a conversion and restoration management. Our results show that there are potentials to restore the acid systems under spruce trees by converting to deciduous tree species, but indicate also that only a change from spruce to deciduous tree species results in a very slow recovery and is maybe not enough to return to the original state. Changing the tree species cover from spruce to deciduous species together with liming showed promising results in the direction of a less acid forest system already after two and a half year. The pH increased in the restoration pathway with lime treatment above the pH of the original deciduous stand without lime addition. Based on the results, it seems that earthworm species and other soil biota are probably also important in the restoration process. However, the earthworms were in the direct neighbourhood of the soil monoliths in this experiment, while the distance for migration in real forest restorations will be much larger. The additional effects of tree species conversion, earthworms and liming in this short-term experiment are promising for full restoration on the long term.

Spruce canopy, Gaume, 2009

7

General discussion & conclusions

Forests and forest management in western Europe have changed during the last two centuries. The proportion of deciduous forests has decreased since the mid-19th century while the proportion coniferous forest has increased. On the one hand, large areas with deciduous forest have been converted to coniferous forest, and on the other hand, large areas of non-forested land were planted with conifers. Since the second half of the 20th century, forest management has become less focused on wood production and the conversions have been slowing down. As the species composition of the forest overstorey has an impact on the chemical, physical and biological characteristics of the soil (Augusto *et al.*, 2002), the conversions to coniferous forest may have a strong impact on the forest ecosystem and its functioning. More recently, reconverting secondary non-native coniferous plantations to more natural deciduous forests has become an important topic in forest management (Mosandl and Küssner, 1999; Spiecker *et al.*, 2004). The general aim of this thesis was to quantify the change trajectories of several abiotic and biotic variables after conversion from deciduous forest to spruce plantations and back. A second aim of this thesis was to get a better insight in the rates of changes of the measured variables along the gradient in buffer capacity.

7.1 Temporal changes in deciduous forests

To gain insight into the temporal changes in the herbaceous understorey of deciduous stands, we first studied 50-year understorey changes in deciduous stands in which the forest management became less intensive (Chapter 2), as in many regions in Europe (e.g. Van Calster *et al.*, 2008; Hedl *et al.*, 2010; Verheyen *et al.*, 2012). The lower management intensity, the abandonment of the coppice with standards management and also the increased acidifying deposition were the main drivers of temporal changes in the understorey diversity and composition in the studied deciduous forests. A new and interesting result was the difference in temporal changes of the understorey vegetation between the site types. The species pool became more than 10 % smaller on the moderately acidic sites and nearly 30 % smaller on the acidic sites. At the moderately acidic sites, plot species richness declined with 20 %, and the community composition shifted towards a higher frequency and abundance of species requiring less light and more nutrients.

7.2 Effects of conversion to spruce

We then focused on conversions from deciduous stands to spruce plantations and back, and studied changes in the forest floor, soil acidity, the earthworm community and understorey vegetation (Chapter 3, 4 & 6). The impact of conversion to spruce on the forest ecosystem was, however, much larger than the management changes in the deciduous stands; all variables measured in this thesis significantly changed after conversion (Chapter 3, 4 & 6). Spruce is indeed generally considered a tree species with strong ecological effects (Ranger and Nys, 1994; Augusto *et al.*, 2002; De Schrijver *et al.*, 2007). The deciduous forests were in a mesotrophic state with a mull-like humus type, soils in the cation exchange buffer range and endogeic earthworm species present. After conversion to spruce, the ecosystem shifted towards an oligotrophic state with moder/mor-like humus types, soils in the aluminium buffer range and a lack of endogeic earthworms (Table 7.1). Most of the results are in line with the expectations based on the ecology of the species. The analysis of all these variables on a single location makes this study innovative and shows interesting links between these variables.

Forest floor

The tree species composition in the deciduous stands in the study area was correlated with the clay content of the soil. On the well-buffered sites, species such as *Acer spp., Fraxinus excelsior* and *Prunus avium* were present, while the stands on the less buffered sites were dominated by *Fagus sylvatica* and *Quercus* spp. (Chapter 2 & 4). The different tree species have different litter qualities resulting in a gradient in forest floor mass, also correlated with the gradient in buffering capacity. For the deciduous stands, the forest floor thickness was about 50 % higher and the forest floor mass was double as high on the acidic sites compared to the less acidic sites (Chapter 2 & 3). The differences between deciduous forest and spruce plantations were even bigger: the mean forest floor mass was three times higher under spruce (Chapter 3, Table 7.1). The chemical composition of leaf litter is an important determinant of the litter decomposition rate: litter rich in calcium and with a low lignin content and C:N ratio decomposes faster (Reich *et al.*, 2005; Hobbie *et al.*, 2006). Leaf litter quality is largely determined by tree species, but site quality can also directly influence litter quality. On sites with higher soil calcium concentrations, the calcium uptake of the trees can be higher (Misson, 2001). Therefore, the litter quality of a species may be better (e.g. due to higher calcium concentrations) on the well buffered soils compared to the more acidic soils.

Soil variables

Slower decomposition causes forest floor build-up and can lead to soil acidification through the production of organic acids as intermediate decomposition products (de Vries and Breeuwsma, 1985) and by causing a delay in the return of buffering cations to the soil where they were taken up by tree roots in exchange for protons (Nilsson *et al.*, 1982). Species-specific dry deposition and canopy exchange can cause differences in potentially acidifying input, with generally a higher potentially acidifying input on the forest floor and soil under evergreen coniferous species compared to deciduous species (De Schrijver *et al.*, 2007). These effects were clearly visible after conversion: the mean pH in the topsoil layer was 0.5 pH units lower under spruce (Chapter 3). In the experimental conversion (Chapter 6), the soil acidification was not visible yet. In the experimental restoration (Chapter 6), we already observed a pH increase after only two and a half years under deciduous tree cover. Studies of temporal changes in understorey vegetation in Belgium frequently observed or inferred a gradual acidification of the soil over time (Van Calster *et al.*, 2007; Baeten *et al.*, 2009a), but this could not be concluded from the variables derived from the understorey vegetation resurvey in this study (Chapter 2).

Earthworm community

Overstorey trees provide strong controls over other biota because of their physical dominance. Trees provide resources for several biota and they influence ecosystem characteristics important for other species (Palik and Engstrom, 1999). Therefore, tree species conversion had a significant influence on, e.g., the earthworm community and the herbaceous understorey layer. There were significant differences between the earthworm community under deciduous and spruce tree cover: the earthworm biomass was five times

Table 7.4. Summary table on the differences between deciduous forest and spruce plantations based on chapters 3, 4 and 6 of this thesis.

	Mixed deciduous forest	**Spruce plantation**
Tree species	*Acer pseudoplatanus, Betula pendula*, *Carpinus betulus*, *Fagus sylvatica*, *Fraxinus excelsior, Quercus petraea* & *Quercus robur*	*Picea abies*
Forest floor (Chapters 3 & 4)	mull-like humus (29/40 plots) forest floor mass: 0.9 kg/m^2	moder/mor-like humus (38/40 plots) forest floor mass: 2.6 kg/m^2 (thick H layer)
Forest understorey vegetation (Chapter 4)	less acid-tolerant species (AIV_R 5.21) less light-demanding species (AIV_L 4.43)	more acid tolerant species (AIV_R 4.24) more light-demanding species (AIV_L 4.79)
	similar mean species richness (19 species/plot) and overall species pool (86 species)	
Decomposers (Chapters 3 & 6)	burrowing earthworms mean endogeic biomass: 16.5 g/m^2	nearly no burrowing earthworms mean endogeic biomass: 1.5 g/m^2
Soil variables (Chapters 3, 4 & 6)	mean topsoil pH-KCl: 3.78 Ca concentration in topsoil: 375 mg/kg	mean topsoil pH-KCl: 3.31 Ca concentration in topsoil: 105 mg/kg

lower under spruce, and there were less epigeic earthworms and nearly no endogeic earthworms present in the spruce stands (Chapter 3). In the conversion and restoration pathway of the experimental study (Chapter 6), the changes in the earthworm community were already significant two and a half year after changing the tree species cover. Tree species influence the earthworm community composition through their leaf litter. Lower calcium concentrations and high C:N in leaf litter will lead to lower earthworm biomass (Reich *et al.*, 2005; Hobbie *et al.*, 2006). The high LAI and continuous leaf cover of spruce also cause dryer soils and high interception rates of potentially acidifying pollutants (Augusto *et al.*, 2002), which adversely affects earthworm populations (e.g. Nordstrøm and Rundgren, 1974; Grossi and Brun, 1997; Wever *et al.*, 2001; Eggleton *et al.*, 2009). Earthworms are sensitive to soil acidification (Nordstrøm and Rundgren, 1974; Edwards, 2004). So, spruce also has an indirect effect on the earthworm community due the increased soil acidity in spruce stands. Burrowing earthworm species contribute to the incorporation of organic matter in the soil and are important to maintain a mull-like humus type (Muys and Granval, 1997). The disappearance of this ecological group in spruce stands due to lower litter quality and acidification of the soil might increase the forest floor build-up and reinforce acidification.

Understorey vegetation

Similar to the overstorey, the species composition of the herbaceous understorey is determined by the site quality. On the well buffered soils, more species and usually also less acid-tolerant species occurred (Ellenberg and Leuschner, 2010). The species richness per plot and the species pool were nearly twice as high on the well buffered sites (Chapter 2), and this was also found in the deciduous and spruce stands where the plot species richness increased in both stands types with increasing clay content (Chapter 4). The changes in the forest management in the study area were partly responsible for the temporal changes in species richness and composition in the deciduous stands, as found by many other studies (e.g. Van Calster *et al.*, 2008; Baeten *et al.*, 2009a; e.g. Hedl *et al.*, 2010). Tree species also had a significant effect on the herbaceous understorey composition due to the physical dominance of trees and their influence on several ecosystem processes, e.g. forest floor build-up and soil acidification, affecting the understorey plants (Barbier *et al.*, 2008). Interestingly, the conversion to spruce did not change the herbaceous understorey diversity. We expected a decrease in species richness due to the conversion to coniferous forest (Barbier *et al.*, 2008) and the mostly lower species richness on more acid soils (e.g. Brunet *et al.*, 1997; Ewald, 2003; Partel *et al.*, 2004). Probably, the higher light availability during the growing seasons compensated for the conversion and the accompanying acidification. The species composition changed after conversion to spruce towards a higher frequency and abundance of more acid-tolerant and light-demanding species. The different tree species had direct effects through their influence on light availability: the AIV_L values changed due to changed forest management and tree species composition in the observatory study (Chapter 2 & 4), and the different light regimes in the pot experiment (Chapter 5) had a significant influence on the plant performance of four of the five herbaceous study species. Several other tree-species-related variables, for example differences in competition for nutrients and throughfall quantity and quality, can influence the understorey vegetation (Barbier *et al.*, 2008). These variables were, however, not measured in this study. We expected a decrease of the AIV_R in the study of the temporal changes in the understorey vegetation (Chapter 2), but found no changes in this value, e.g. probably due to the strong correlation between AIV_R and AIV_N. By contrast, in the comparison of spruce and deciduous stands (Chapter 4), we found a strong decrease of the AIV_R and no change in the AIV_N. In this study, we also directly measured the changes in soil acidity: the differences between spruce and deciduous stands were clearly significant, and certainly larger than the temporal changes in soil pH in the deciduous stands. Thus, the AIV_R can be useful in case of strong changes in acidity, but is probably not sensitive enough or overruled by other effects in case of small changes in soil pH. Besides direct effects, also indirect effects occurred. The presence of spruce caused forest floor build-up and soil acidification, which resulted in more acid-tolerant species in the understorey vegetation. The germination of understorey species was also influenced by the tree species: the germination index for *Luzula luzuloides* was twice as high in soil originating from deciduous stands compared to soil from spruce stands, and there was nearly no germination for *Primula elatior* in the spruce soil (Chapter 5). Hence, both the lower germination and the lower adult performance of several forest understorey species under spruce may have influenced the understorey composition in the spruce stands.

7.2.1 Rates of change along the soil buffering gradient

In our study area, both soils in the cation exchange buffer range ('well buffered') and soils near or in the aluminium buffer range ('more acidic soils') occurred. All observations of the three observational chapters (2, 3 & 4) and the first experimental chapter (5) were done along the entire gradient of soil buffering capacity and brought forward innovative results in line with our expectations. The changes in soil variables, earthworm, understorey and seed bank community between deciduous and spruce stands differed along the soil buffering gradient, with larger changes on the well buffered soils. In the vegetation resurvey of chapter 2, we distinguished two groups of plots based on the vegetation composition. The temporal changes in Ellenberg indicator values and species richness were only significant for the less acidic sites. In chapters 3, 4 and 5, we used the clay content of the soil as a proxy for the buffering capacity of the soil. The differences in pH and earthworm biomass between deciduous and spruce stands were several times larger on the well buffered soils (Chapter 3). The results of chapter 4 showed a correlation between the changes in Ellenberg indicator values for acidification (AIV_R) after conversion and the soil buffering gradient. The difference between deciduous and spruce stands in the number of species in the seed bank was also correlated with the pH-gradient (Box 2). Opposite to our expectations, we did not find differences along the soil buffering gradient in our (re)conversion experiment (Chapter 6), probably due to the short time period of the experiment and the limited number of samples along the gradient.

We assume that the following three interrelated causes contributed to the differences in rates of change along the gradient in buffer capacity: the difference in buffer system, the changes in the earthworm community and the differences in litter quality. (i) Soils can buffer incoming protons with a series of buffer reactions along the soil pH gradient (Bowman *et al.*, 2008). The capacity of a soil to buffer incoming protons depends on the buffer system (Box 1). Since we can assume that the acidifying input in the spruce stands was similar along the soil buffering gradient, the difference in topsoil pH between deciduous and spruce stands is expected to be larger at the well buffered sites that are still in the cation exchange buffer range, since this is a small and quickly exhausted buffer range (Box 1). (ii) The higher earthworm mass and the presence of endogeic earthworm species at the well-buffered sites probably contributed to the larger differences between deciduous and spruce stands on these sites. Endogeic species will disappear when the soil reaches the aluminium buffer range. On the well buffered soils, endogeic earthworms were numerously found in the deciduous stands, while we could not detect them in the spruce stands. The differences in earthworm biomass within a plot pair were smaller at the more acid sites, and there were no endogeic species in both the deciduous and spruce stands at these sites. The bigger difference in earthworm biomass between deciduous and spruce stands on less acid sites most likely lead to differences in the soil variables and forest floor because of the importance of earthworms in maintaining a good humus quality and, more generally, as ecosystem engineers (e.g. Jones *et al.*, 1994; Barot *et al.*, 2007). (iii) The composition of the deciduous forest matrix changed along the buffering gradient: on the well-buffered sites more species with fast-decomposing litter such as *Acer* spp*.*, *F. excelsior* and *P. avium* occurred, while the stands on the sites with lower buffering capacity were dominated by oak and beech, tree species with slowly-decomposing litter (Chapter 2). The difference in leaf litter quality between oak or beech and spruce is smaller than between species such as *F. excelsior* en *P. avium* and spruce (Hobbie *et al.*, 2006; Vesterdal *et al.*, 2008). These bigger differences in litter quality on well-buffered soils caused larger effects of conversion on the soil, forest floor and earthworm community.

7.2.2 Restoration potential

Many spruce stands in the study region and at several other places in Europe are at an economically interesting age for harvesting; the oldest converted stands in our study area have already been cut. Therefore, it is highly relevant to look into the potential for restoring the oligotrophic spruce plantations back to mesotrophic deciduous forests. Integrated restoration with a combination of abiotic (neutralisation of the acidification by liming) and biotic restoration measures (e.g. conversion to tree species with fast decomposing litter and increase of the earthworm community) can possibly increase the chances for successful restoration (Muys *et al.*, 2003). In our short-term experiment, we found a potential for integrated restoration (Chapter 6). After two and a half years, the soil pH and the earthworm biomass had already increased and the forest floor biomass had decreased. However, we could not find differences between the length of the change trajectory in the conversion and the restoration cases. The changes in the conversion pathway seemed to be slightly smaller than in the reconversion pathway. Possibly, the deciduous stand monoliths were temporarily buffered against the higher acidifying input and lower litter quality in the spruce stands and could therefore temporarily stay at a similar state. On the other hand, the better litter quality, the earthworms and the accelerated litter decomposition in the restoration pathway seemed to have positive effects on the restoration of the spruce monoliths. The abiotic conditions can be restored, but it remains unclear whether this will lead to restoration of the herbaceous understorey vegetation. We found in the observational study of the seed bank (Box 2) that the species characteristic for the deciduous stands were mostly absent in the seed bank of both stand types. Colonisation of these species is generally very slow which will limit the fast restoration of the understorey.

7.3 Suggestions for further research

We measured trajectories of change after conversion for several variables in this thesis. The data set may be extended with other variables, for example measurements on bryophytes, other soil and forest floor fauna as well as fungi could be taken. Bryophytes are highly indicative for the conditions of the topsoil layers and canopy and were abundant in most spruce stands but nearly absent in deciduous stands. The role of roots and soil processes in relation to tree species effects are also less understood and therefore deserve more attention.

An interesting point of this study was the study of several variables (soil, forest floor, earthworms and understorey vegetation) along a soil buffering gradient in one region. This study showed that there were many and complex relations between these variables, but it was not always clear how the variables interacted with each other and whether feed-back mechanisms were present. We have tried to apply statistical tests that allow testing causal relationships between variables. We tried to test a causal model between the measured variables using, for example, structural equation models, but our data set did not have enough replications and data on some variables were missing (for example measurements of the leaf litter quality) for getting satisfying and correct results, and probably supplementary and specific experiments are necessary.

Another question that still remains is how the change trajectories develop over time: along a gradual change trajectory, a non-hysteresis threshold model, or a hysteresis threshold model (Fig. 7.1, Suding and Hobbs, 2009). Gradual changes occur when there is a linear relationship between the environmental conditions and the ecosystem state. Non-hysteresis threshold models occur when the state of the ecosystem rapidly changes at a certain point on the environmental gradient. Hysteresis thresholds can occur if there are multiple ecosystem states within the same habitat (alternative stable states sensu Scheffer *et al*., 2001); the environmental threshold conditions at which state one declines and shifts to the second state differs from the threshold conditions at which state two changes to state one. In our study system, there are two clearly different states: a mesotrophic state in the deciduous stands and an oligotrophic state after conversion in the spruce stands (Table 7.1), and no real gradient between them (cf. Fig. 7.1 b & c). Unfortunately, we did not have measurements along the whole age range of the spruce stands to study the complete trajectory of change. Yet, the complex interactions and feedback mechanisms between the variables measured in our study, in combination with the two distinct ecosystem states, lead us to expect a threshold model situation in our study area (Fig. 7.1 b or c).

According to Scheffer *et al.* (2001), the combination of the following conditions gives a strong indication of the presence of alternative stable states: (1) a shift from one state to another is preceded by a hardly observable decrease of resilience of the original state; (2) severe disturbances trigger shifts between states, facilitated by feed-forward mechanisms; (3) there is a clear contrast between states, and (4) restoring the original state is difficult because the restoration efforts have to tilt the system with a combination of both biotic and abiotic levers. Evidence for alternative stable states should come from mechanistic models validated with experimental data (Scheffer and Carpenter, 2003). Further analysis of our data in combination with data from other studies and long-term restoration experiments is necessary to find evidence for regime shifts. The first condition of Scheffer *et al.* (2001) might be demonstrated by studying also younger spruce plantations, between the time of conversion and the youngest stands in our study (35 years old). The second condition is something we expected but were not able to prove: several changes reinforce each other. For example, the earthworm biomass may have decreased because of the lower litter quality and the acidification of the soil, but the lower earthworm biomass also caused further forest floor build-up and acidification of the soil. The third condition clearly stands in our study; we found clear differences between the deciduous and spruce ecosystem states. An experiment like the one in chapter 6 can be used to proof the fourth condition, but measurements on several time steps and during a longer time period are needed.

Monitoring conversions *in situ* can give us information about the possibilities for ecological restoration in real forest conditions. The soil monoliths in the experiment (Chapter 6) were small, which made colonisation by earthworms possible; earthworm migration may be more difficult in real conversion cases. The time period of this experiment was also limited, but sufficiently long to give us some first insights. The

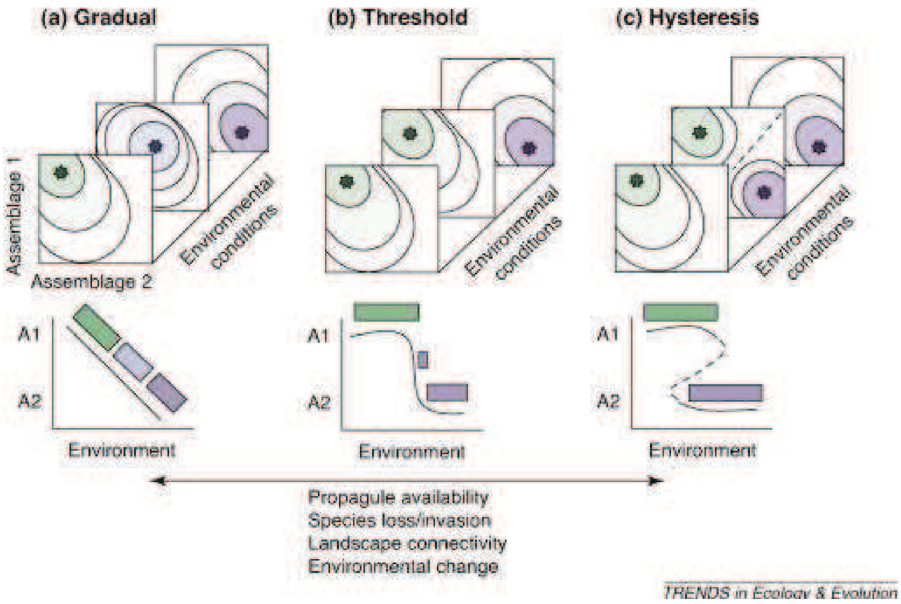


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Figure 7.1. Alternative models of ecosystem dynamics: gradual change (a), and two threshold models: non-hysteresis (b) and hysteresis (c). Each square defines relative abundances of two state characters (e.g. different species, functional groups or ecosystem processes). The ovals represent isoclines of perturbation strength (resilience) and the stars represent attractors. The dotted line in (c) indicates boundaries of basins of attraction. (From: Suding and Hobbs, 2009).

experiment was an approach to attempt the effects of conversion, but in real forest conversions there are several additional disturbances and there is a period with succession between the young planted stand and the full grown forest stand. Foresters and scientists could also experiment in these stands with integrated restoration and test different conversion scenarios. We studied the effects of ecological restoration on the soil, forest floor and earthworm community, but studying the effects of different restoration scenarios on other variables, for example the understorey vegetation, may be interesting as well.

We resurveyed a more than fifty year old vegetation survey in deciduous stands. These data gave us interesting information about long-term changes in the forest understorey. The old and recent data sets can be used for a further monitoring of the changes in the forest understorey in the future. The data from chapter 3 and 4 can also be used in a follow-up study on the spruce stands or on restoration pathways when the stands are reconverted.

7.4 Management implications

This thesis showed that the conversion from deciduous forest to spruce plantations changed several characteristics of the forest ecosystem. The soil, forest floor, earthworm community and vegetation changed from a mesotrophic to an oligotrophic state. Managers have to be aware that changing the tree species composition and management system also changes the communities of other species groups and ecosystem processes.

Because of the equal species richness but divergent species composition in both stand types, the islands of spruce plantations in the deciduous forest matrix lead to a larger overall species pool and a higher across-site β diversity, increasing the biodiversity at the scale of our study region (Chapter 3). Yet, many of the recorded understorey species in the spruce stands were relatively light-demanding species that also occur in open habitats or deciduous forest edges and are less interesting from a biodiversity conservation point-of-view. At the regional scale, the more acid-tolerant vegetation of the spruce stands is very common in the extensive spruce forests of the adjacent Ardennes and many other low mountain areas with non-calcareous bedrock in western and central Europe. The number of red-list species was also lower in the spruce stands compared to the deciduous stands. Moreover, across Belgium and Europe, mesotrophic deciduous stands with a well-developed understorey layer like in our study area are quite rare. The study of the temporal changes in the deciduous forest (Chapter 2) showed that we are losing species in these forests and that several typical ancient-forest species seems to disappear. These deciduous forest types (e.g. 9110, *Luzulo-Fagetum* beech forests and 9160, sub-Atlantic and medio-European oak or oak-hornbeam forests of the *Carpinion betuli*) are habitattypes described for conservation in the annexes of the EU Habitat Directive (Council Directive 92/43/EEG, 1992) and, in contrast with spruce plantations, important to protect on an European scale. So, although at first sight conversion does not seem bad for the understorey diversity, the deciduous-to-spruce conversions in the study area were negative for the herbaceous understorey, especially on the well buffered soils.

The impact of the conversions is mostly local and changed mainly variables in the converted stands. In the study area, the Gaume region, the impact on the regional forest system was rather small because the islands are mostly small and the total area with spruce plantation is limited. Therefore, the impact of the conversions on the forests on a regional scale was rather small. Yet, new conversions are not recommended because of the large impact on the soil chemistry, soil biota and several other ecosystem variables. On the well buffered soils, we have to be even more careful with conversions because of the higher impact on these sites. The mesotrophic deciduous stands with a well-developed understorey layer are quite rare and are also threatened by atmospheric deposition. In addition, spruce plantations are vulnerable to abiotic and biotic damages (Spiecker, 2000; Solberg, 2004). In the study area, spruce was planted outside its distributional limits, which involves risks as climate change may render the zone unsuitable for spruce (Bradshaw *et al.*, 2000).

Managers will have to decide what to do with the current spruce stands. Since the survey of 2009, some spruce stands have already been clearcut and several other stands are near the age of final cut. We expect that replanting spruce trees will cause further acidification of the sites due to the permanently high acidification potential of spruce. Conversion to spruce did already lower the soil buffer capacity; the impact of a second generation spruce will likely be smaller. Restoration to mixed deciduous stands, i.e., changing the tree cover to deciduous trees, will probably take very long and may even not be enough to get a full ecological restoration of the forest ecosystem to the pre-conversion state. Due to the larger impact of conversion on the well buffered soils, we expect the restoration to be more difficult on these sites. Implementing an integrated restoration, i.e., using tree species with good litter quality and adding lime, will yield a greater chance of full ecological restoration (Chapter 6).

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Curriculum vitae

Personal data

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Date of birth	20 October 1985
Nationality	Belgian
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Education

2006–2008	MSc in Bioscience engineering, Land & Forest Management Ghent University, Faculty of Applied Biological Sciences
2003–2006	BSc in Bioscience engineering Ghent University, Faculty of Applied Biological Sciences
1997–2003	Secondary School (Mathematics-Sciences) Sint-Annacollege, Linkeroever, Antwerpen

Professional experience

January – present	Project Coordinator at Natuurpunt – LIFE+ project ‘Nature restoration in Vochtig Haspengouw’
November 2008-October 2012	PhD research at Ghent University, Faculty of Bioscience Engineering, Department of Forest and Water Management, Forest and Nature Lab

Scientific publications

Publications in international journals with peer review cited in the Science Citation Index

Submitted

Verstraeten G., Baeten L., De Frenne P., Thomaes A., Demey A., Muys B. & Verheyen K. Forest herbs show species-specific responses to the variation in light regime and soil acidity: an experiment mimicking forest conversion scenarios. Basic and Applied Ecology.

Verstraeten G, De Schrijver, A, Hlava J, Wuyts K, Schelfhout S, Verheyen K, Muys B. The biogeochemical effects of deciduous to coniferous forest conversion differ along a gradient of soil buffering capacity. Soil Biology Biochemistry.

Published

Thomaes A., De Keersmaeker L., De Schrijver A., Baeten L., Vandekerkhove K., Verstraeten G. & Verheyen K. (in press). Can soil acidity and light availability help to explain tree species effects on forest herb layer performance in post-agricultural forests. Plant and Soil. doi 10.1007/s11104-013-1786-x

Demey A., Ameloot E., Staelens J., De Schrijver A., Verstraeten G., Boeckx P., Hermy M. & Verheyen K. (2013). Effects of two contrasting hemiparasitic plant species on biomass production and nitrogen availability. Oecologia, 173, 293-303.

Verstraeten G., Baeten L., De Frenne P., Vanhellemont M., Thomaes A., Boonen W., Muys B. & Verheyen K. (2013). Understorey vegetation shifts following the conversion of temperate deciduous forest to spruce plantation. Forest Ecology and Managment, 289, 363-370.

Verstraeten G., Baeten L., Van den Boeck T., De Frenne P., Demey A., Tack W., Muys B. & Verheyen K. (2013). Temporal changes in forest plant communities at different site types. Applied Vegetation Science, 16, 237-247.

De Schrijver A., De Frenne P., Staelens J., Verstraeten G., Muys B., Vesterdal L., Wuyts K., Van Nevel L., Schelfhout S., De Neve S. & Verheyen K. (2012). Tree species traits cause divergence in soil acidification during four decades of postagricultural forest development. Global Change Biology, 18, 1127-1140.

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.

Verheyen K., Baeten L., De Frenne P., Bernhardt-Roemermann M., Brunet J., Cornelis J., Decocq G., Dierschke H., Eriksson O., Hedl R., Heinken T., Hermy M., Hommel P., Kirby K., Naaf T., Peterken G., Petrik P., Pfadenhauer J., Van Calster H., Walther G.-R., Wulf M. & Verstraeten G. (2012). Driving factors behind the eutrophication signal in understorey plant communities of deciduous temperate forests. Journal of Ecology, 100, 352-365.

Baeten L., Verstraeten G., De Frenne P., Vanhellemont M., Wuyts K., Hermy M. & Verheyen K. (2011). Former land use affects the nitrogen and phosphorus concentrations and biomass of forest herbs. Plant Ecology, 212, 901-909.

Verstraeten G., Baeten L. & Verheyen K. (2011). Habitat preferences of European nightjars *Caprimulgus europaeus* in forests on sandy soils. Bird Study, 58, 120-129.

Publications in national journals with peer review

De Schrijver A., Wuyts K., Schelfhout S., Staelens J., Verstraeten G. & Verheyen K. (2012). Verzuring van terrestrische ecosystemen: oorzaken, remedies en gevolgen voor de biodiversiteit. Natuur.Focus, 11, 136-143.

Scientific reports

Verheyen K., De Schrijver A., Staelens J., Baeten L., De Frenne P., Adriaenssens S., Verstraeten G., Ampoorter E., Van Nevel L., Demey A., Wuyts K. & Gruwez R. (2010). Pilootstudie naar kwantificering van de relaties tussen de achteruitgang van biodiversiteit en chronische overschrijding van kritische lasten: eindrapport

Abstracts of presentations at scientific congresses

Verstraeten G., Baeten L., Muys B. & Verheyen K. (2012). Compositional shifts in the understorey vegetation after the conversion from mixed deciduous forest to spruce monocultures. Abstract of oral presentation at 2nd International conference on Biodiversity in Forest Ecosystems and Landscapes. (Cork, Ireland, 27-31 August 2012)

Verstraeten G., Baeten L., Muys B. & Verheyen K. (2012). Veranderingen in de kruidlaag na omvorming van gemengd loofbos naar monoculturen van fijnspar. Abstract of poster presentation at Starters in het Natuur- en Bosonderzoek. (Brussel, Belgium, 16 March 2012)

Sluijs S., Verstraeten G., Bonte D. & Verheyen K. (2012). Effecten van boomsoortgeïnduceerde verzuring op de zaadbank. Abstract of poster presentation by S. Sluijs at Starters in het Natuur- en Bosonderzoek. (Brussel, Belgium, 16 March 2012)

Verheyen K, Baeten L, De Frenne P, Verstraeten G (2011) It's more than nitrogen deposition: driving factors behind the eutrophication signal in temperate forest understorey plant communities. Abstract of oral presentation given by K Verheyen at the Annual Symposium of the British Ecological Society. (Cambridge, UK; 28 – 30 March 2011)

Verstraeten G., Baeten L., Muys B. & Verheyen K. (2011). Compositional shifts in the understorey vegetation after the conversion from mixed deciduous forest to spruce monocultures. Abstract of poster presentation at Young Botanist Day. (Leuven, Belgium, 2 December 2011)

Verstraeten G., Hlava J., Muys B. & Verheyen K. (2011). Soil, litter and herb layer changes after conversion to spruce monocultures. Abstract of oral presentation at the 41st annual meeting of the ecological Society of Germany, Austria and Switzerland. (Oldenburg, Germany, 5 – 9 September 2011).

Verstraeten G., Hlava J., Muys B. & Verheyen K. (2011). Veranderingen in bodem- en strooiselkenmerken bij de omvorming van gemengd loofhout naar fijnspar. Abstract of oral presentation at Starters in het Bosonderzoek. (Brussel, Belgium, 17 March 2011)

Verheyen K, Baeten L, De Frenne P, Verstraeten G (2010). Long-term understory vegetation changes in northwestern European forests: a synthesis of (semi-) permanent plot studies. Abstract of oral presentation given by K Verheyen at XXIIIth IUFRO World Congress (Seoul, Republic of Korea; 23 – 28 August 2010)

Muys B., Verstraeten G., Hlava J., Valckx J. & Verheyen K. (2010). Change trajectories of earthworm communities after conversion of mixed semi-natural broadleaved forest to spruce monoculture along a soil trophic gradient. Abstract of oral presentation given by B Muys at the 9th International symposium on Earthworm Ecology. (Xalapa, Mexico, 5-10 September 2010)

Verheyen K, Baeten L, De Frenne P, Verstraeten G (2009). Long-term vegetation changes in northwestern European forests: a synthesis of (semi-)permanent plot studies. Abstract of oral presentation given by K Verheyen & L Baeten at Symposium of British Ecological Society (Cambridge, UK; October, 2, 2009)

Verstraeten G. & Verheyen K. (2009). Habitatvoorkeur van de nachtzwaluw (*Caprimulgus europaeus*) in het gewestbos Pijnven te Hechtel-Eksel en implicaties voor het beheer. Abstract of poster presentation at Starters in het Bosonderzoek. (Brussels, Belgium, 19 March 2009)

MSc thesis

Verstaeten G (2008) Habitatvoorkeur van de nachtzwaluw (Caprimulgus europaeus) in het gewestbos Pijnven te Hechtel-Eksel en implicaties voor het beheer. MSc thesis, Ghent University, Ghent, Belgium

Scientific activities

Participation in congresses, symposia or workshops

Participation with oral presentation

27 – 31 August 2012 Verstraeten G., Baeten L., Muys B. & Verheyen K. Compositional shifts in the understorey vegetation after the conversion from mixed deciduous forest to spruce monocultures. Biodiversity in Forest Ecosystems and Landscapes, 2nd International conference, Cork, Ireland.

5 – 9 September 2011 Verstraeten G., Hlava J., Muys B. & Verheyen K. (2011). Soil, litter and herb layer changes after conversion to spruce monocultures. 41st Annual Meeting of the Ecological Society of Germany, Austria and Switzerland, Oldenburg, Germany.

17 March 2011 Verstraeten G., Hlava J., Muys B. & Verheyen K. Veranderingen in bodem- en strooiselkenmerken bij de omvorming van gemengd loofhout naar fijnspar. Starters in het Bosonderzoek, Brussels, Belgium.

Participation with poster presentation

19 March 2009. Habitatvoorkeur van de nachtzwaluw (*Caprimulgus europaeus*) in het gewestbos Pijnven te Hechtel-Eksel en implicaties voor het beheer. Starters in het Bosonderzoek, Brussels, Belgium

2 December 2011 Verstraeten G., Baeten L., Muys B. & Verheyen K. Compositional shifts in the understorey vegetation after the conversion from mixed deciduous forest to spruce monocultures. In: Young Botanist Day, Abstracts.

16 March 2012 Verstraeten G., Baeten L., Muys B. & Verheyen K. (2012). Veranderingen in de kruidlaag na omvorming van gemengd loofbos naar monoculturen van fijnspar. In: Starters in het Natuur- en Bosonderzoek, Abstracts.

Participation without presentation

23 – 24 November 2011 International Conference Forests 2011. Leuven, Belgium

23 September 2011 Ecologische bosuitbreiding op sterk bemeste gronden: van wetenschap naar praktijk. Brussel, Belgium.

7 – 8 Februari 2011 The Netherlands annual ecological meeting. Lunteren, Netherlands.

8 – 10 September 2010 Biodiversity in a changing world. Ghent, Belgium.

1 – 6 July 2010 Youth perspective conference on Biodiversity. Antwerp, Belgium.

21 – 23 June 2010 Workshop Introduction in Structural Equation Modelling. Bologna, Italy.

6 May 2008 Workshop Modelling for conservation. Brussels, Belgium

23 November 2006. Van dennenplantages naar een beloofd land: Theoretische en praktische aspecten van bosomvorming. Hasselt, Belgium.

Supervision of MSc thesis students

2011–2012 Silas Sluijs Effecten van boomsoortgeïnduceerde verzuring op de zaadbank: het potentiële herstel van de kruidlaag na omvorming van fijnsparbestanden (*Picea abies*) naar loofbos. Supervisor: Prof. dr. ir. Kris Verheyen