Supervisors:	Prof. Dr. ir. Kris Verheyen
	Ghent University, Department of Environment, Forest & Nature Lab
	Dr. Michael P. Perring
	Ghent University, Department of Environment, Forest & Nature Lab
Dean:	Prof. Dr. ir. Mark Van Meirvenne
Rector:	Prof. Dr. ir. Rik Van de Walle

HABEN BLONDEEL

Disentangling effects of past land use and global environmental change on temperate forest understorey communities

THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR (PHD) OF BIOSCIENCE ENGINEERING:
NATURAL RESOURCES

Dutch translation of the title:

Het ontkoppelen van historische landgebruikseffecten en globale milieuveranderingen op kruidlaaggemeenschappen in gematigde bossen

Illustration on the cover:

Front: *Hyacinthoides non-scripta* (Sofie Verstraelen)

Back: Plot with Light treatment in Aelmoeseneiebos, Gontrode (Sofie Verstraelen)

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Image: Sofie Verstraelen

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Image: Haben Blondeel

Summary

This PhD thesis aimed to unravel the relative importance of past land use and multiple environmental changes on development of understorey communities in temperate forest.

The forest understorey can be defined as the ground layer of forest vegetation with a high abundance of herbaceous species, which can represent up to 90% of the plant species richness in temperate forests. The understorey can thus be important to many ecosystem functions, for instance to nutrient cycling. Furthermore, these functionally rich communities can have an important "early-warning" potential for forest responses to global environmental change. One of the key drivers for change in the understorey is the land-use history of the forest. Past agricultural land use can influence the composition of understoreys for centuries. Past agricultural land use can have persistent legacies in the soil, such as enrichment in phosphorus (P) due to prior fertilisation. Important contemporary environmental change drivers that alter resources and conditions in temperate forests are climate change, nitrogen (N) deposition and changing forest management. To disentangle these drivers on understorey community development, we designed and performed a large field experiment. We applied *in situ* two-level full factorial treatments of temperature, light availability (representing changing forest management), and N availability on herbaceous plant communities that grew on soils with a distinct land-use history. This thesis progressively describes these altered resources and conditions, followed by individualistic plant responses to these alterations, which in turn could scale up to a plant community response.

We collected soil from 24 pairs of ancient forest (in existence before 1850) and post-agricultural forest (established around 1950 on former arable land) across eight European regions (Chapter 2). These eight European regions varied along gradients of soil types, N deposition and agricultural intensity. Combining soil data from the paired sites of ancient and post-agricultural forests along these gradients successfully elucidated consistent legacies of past land use (Chapter 2). As expected, post-agricultural forest soil had an overall higher available phosphorus (P) concentration, higher pH, and lower carbon to nitrogen (C:N) ratio compared to ancient forest. The magnitude of these legacies varied along gradients of soil types, P nutrient management on the regional level, and N deposition (Chapter 2).

We created 384 "mesocosms" by planting mixtures of slow and fast colonising plant species on these ancient and post-agricultural soils (Chapter 3). We planted communities of five species (four individuals per species), which were assembled from a pool of 15 forest herb species with varying ecological strategies. We can thus understand understorey development in response to past land use while bypassing dispersal limitation in species. We applied two-level full factorial treatments of enhanced light availability (using fluorescent tubes), warming (using open top chambers), and N addition (by fertilisation with NH_4NO_3). About half of the original 7680 individuals survived to the next year, but with large variation in mortality between species (Chapter 3).

We measured functional traits of the species in the mesocosm, by accounting for plant height and resource acquisitive traits (i.e. leaf traits). Important leaf traits are either morphological, such as the leaf area to leaf mass ratio (specific leaf area, SLA), or physiological such as leaf N content. We expected that these functional traits would respond individualistically to the environmental treatments, with a potentially accelerating response (i.e. positive interaction) due to an agricultural land-use history, and depending on a species' ecological strategy. We found that fast colonisers were tall with large N uptake, which is a functional signature that points towards resource acquisition (Chapters 4 and 5). Contrastingly, slow colonising and typical woodland flora lacked in physiological responses (changing leaf N), in favour of leaf morphological responses (changing SLA). Individualistic species responses occurred directly in response to specific manipulations in resources and conditions that benefited a species, and were indirectly exacerbated by higher community productivity (Chapters 4 and 5). Warming and illumination reduced leaf N content of individuals directly, likely due to changing N allocation patterns. Warming and illumination increased vegtetation cover, which further reduced leaf N content. This indirect effect of environmental change, via vegetation cover, was three times less strong then direct physiological responses. These results imply a tissue dilution of N with increasing growth, in response to environmental change directly and indirectly exacerbated by community cover (Chapter 5). We barely found interactive treatment effects, so that combined effects of environmental treatments were additive and characterised by the sum of the separate treatment effects.

To scale the individualistic responses of plant species to the community level, we characterised abundance changes (i.e. cover) of species due to treatments over time and associated this community change to responses of functional traits on the community level. We found that light, then warming, were the consistent drivers for diverging plant community trajectories in our mesocosm experiment. The N enrichment treatment, like the forest land-use history, had limited effects on community organisation. Communities reordered towards taller plants with illumination and warming, while spring geophytes hold steady in unlit conditions (Chapter 6). Change in community plant height could thus be estimated by accounting for interspecific differences of plant height in forest herb populations. However, to scale SLA from individuals to the community, variation in growing environments and associated individualistic responses needed to be taken into account (Chapter 4, Chapter 6). Intraspecific trait variation (ITV) is consequently key to understand SLA change in communities over time.

Our findings imply that subtle differences in light availability and air temperatures can yield relatively large community trait responses to global change on the short-term, both within and between forest herb species. Against our expectations, these experimental results after three years of monitoring showed a limited importance of N enrichment and the forest land-use history on development of herbaceous understorey communities. It might thus be safe to assert that plant growth on these soils were less limited by N and P availability, and more by light availability and growing temperatures in early spring. The lack of nutrient enrichment effects on the short-term does not imply that such interactive effects are not important to understorey community development in response to global change. It rather shows the complementarity of experimental research to long-term vegetation resurveys or mechanistic modelling approaches, where such effects are observed. More time in the experiment could reveal whether diverging community trajectories in response to environmental change would emerge depending on the forest land-use history in the future.

The findings that we have presented here can contribute to the field of global change ecology. Incorporating ITV on top of species reordering can improve predictions of future plant communities, while acknowledging that subtle differences in light availability can yield large community trait responses to global change. Further research could explore plant-soil interactions in response to these subtle environmental changes, with a strong focus on forest ecosystem functioning in the future. Our current findings provide perspectives for adaptive management to global change, and demonstrates that dimming light availability is key for understorey restoration in a changing world. These perspectives are relevant for policy makers across the globe that have committed to forest restoration for mitigating global change. Ecological research could thus inform policy makers for the benefit of nature and human well-being, especially in the light of the upcoming United Nations Decade of Ecosystem Restoration (2021 - 2030).

Samenvatting

Dit doctoraatsonderzoek heeft als doel om de relatieve invloed van historisch landgebruik en meerdere veranderingen in leefmilieu te achterhalen op de ontwikkeling van plantengemeenschappen in de kruidlaag van bossen.

De kruidlaag kan gedefinieerd worden als de onderste vegetatielaag in bossen. Hier vind je het grootste aantal plantensoorten in het bos met een hoog aantal kruidachtigen, die samen tot 90% van de totale plantensoortenrijkdom in gematigde bossen kunnen vertegenwoordigen. De kruidlaag kan bijgevolg belangrijk zijn voor het functioneren van het bosecosysteem, bijvoorbeeld voor het behouden van efficiënte nutriëntencycli. Bovendien kunnen deze plantengemeenschappen een belangrijk signaal geven wanneer het leefmilieu wijzigt, net doordat er zoveel verschillende types plantensoorten in voorkomen. Een belangrijke verandering in het leefmilieu kan worden voortgebracht door een voormalig landbouwgebruik van de bosbodem. Dit historisch landgebruik kan het bos gedurende eeuwen aantasten met langdurige invloeden op de soortensamenstelling van de kruidlaag, bijvoorbeeld door aanhoudende aanrijking in fosfor (P) via voormalige bemesting. Pertinente huidige wijzigingen in het leefmilieu van bossen zijn klimaatverandering, stikstof (N) depositie, en een veranderend bosbeheer dat het lichtregime aantast. Om de effecten van deze veranderingen op de kruidlaagontwikkeling te ontkoppelen van mekaar hebben we een grootschalig veldexperiment opgezet. We hebben *in situ* behandelingen toegepast met temperatuurswijzigingen, lichttoediening (dat het wijzigend bosbeheer voorstelt), en beschikbaarheid in stikstof op kruidlaaggemeenschappen die groeiden op bodems met een verschillend historisch landgebruik. Dit doctoraatsonderzoek beschrijft de gewijzigde grondstoffen en condities die deze veranderingen in het leefmilieu hebben voortgebracht, gevolgd door individuele plantenresponsen op deze wijzigingen, die op hun beurt opgeschaald kunnen worden naar de plantengemeenschap in zijn geheel.

We verzamelden bodem van 24 paren oud bos (al bos voor 1850) en bos met een recent landbouwverleden (sinds ca.1950 gevestigd op voormalige akkers) in acht Europese regio’s (Hoofdstuk 2). Deze acht Europese regio’s varieerden langsheen gradiënten van bodemtypes, N depositie en landbouwintensiteit. Het combineren van deze bodemgegevens uit paren van oud bos en bos met een recent landbouwverleden kon consistente effecten van het historisch landbouwgebruik op de bosbodem verhelderen (Hoofdstuk 2). Zoals verwacht vonden we een hogere bodemconcentratie in fosfor (P), een hogere pH en een lagere koolstof tot stikstof verhouding (C:N) in bossen met een recent landbouwverleden. De grootte van deze effecten hing af van de gradiënten in landbouwintensiteit, N depositie en bodemtypes (Hoofdstuk 2).

We maakten 384 “mesocosms” door zowel traag- als snel koloniserende plantensoorten te planten op de bosbodems met en zonder een voormalig landbouwgebruik (Hoofdstuk 3). We plantten gemeenschappen van vijf soorten (vier individuen per soort), die verzameld werden uit een poel van 15 soorten met verschillende ecologische strategieën. Met deze methode kunnen we de ontwikkeling in de kruidlaag opvolgen door toedoen van het voormalig landgebruik, zonder dat soorten zich spontaan hoefden te vestigen (waar er veel variatie op kan zitten door verbreidingslimitatie). We pasten behandelingen toe in alle combinaties van lichttoediening (met behulp van Tl-lampen), opwarming (met kleine groeikamers met een open top) en stikstofaanrijking (met bemesting van NH_4NO_3). Ongeveer de helft van de oorspronkelijk 7680 individuen hebben het eerste jaar overleefd, maar met een groot verschil tussen de soorten (Hoofdstuk 3).

We hebben functionele plantenkenmerken van de soorten opgemeten in de mesocosms, door planthoogte en bladkenmerken in rekening te nemen. Belangrijke bladkenmerken kunnen morfologisch zijn, zoals de verhouding van bladoppervlakte tot bladgewicht (specifieke blad oppervlakte, SLA), of fysiologisch zoals het stikstofgehalte (blad N). We verwachtten dat deze functionele kenmerken zouden reageren op de verschillende behandelingen op een individualistische manier binnen de soorten, afhankelijk van de ecologische strategie van een soort. We vonden dat snelle kolonisatoren groter waren en een grotere stikstofopname in bladeren hadden, wat wijst op een “gulzig” gebruik van grondstoffen (Hoofdstukken 4 en 5). Trage kolonisatoren en typische oudbosplanten hadden daarentegen weinig fysiologische responsen (veranderingen in blad N) ten voordele van morfologische veranderingen (veranderen van SLA). Deze individualistische responsen gebeurden direct als functie van meerdere behandelingen, en waren bovendien indirect versterkt door veranderingen in productiviteit van de gemeenschap. In het geval van N opname in bladeren hadden opwarming en belichting drie keer grotere negatieve effecten op blad N gehalte en N opname dan indirecte negatieve effecten via een toenemende productiviteit. Deze resultaten impliceren een verdunning van N in weefsels bij toenemende groei, in respons tot de veranderingen in leefmilieu en indirect versterkt door een toenemende vegetatiebedekkeing (Hoofdstuk 5). We vonden nauwelijks interactieve effecten tussen behandelingen, zodat gecombineerde effecten van behandelingen additief waren en kunnen beschouwd worden als de som van de aparte behandelingen.

Om de individualistische responsen te kunnen opschalen naar het gemeenschapsniveau hebben we veranderingen in abundantie (i.e. bedekking) van soorten ingeschat doorheen de tijd, en deze wijzigingen gekoppeld aan de responsen van functionele plantenkenmerken. We vonden dat lichttoediening, gevolgd door opwarming, de belangrijkste wijzigingen in het leefmilieu waren die een respons van de gemeenschappen veroorzaakten (Hoofstuk 6).

Aanrijking van N was, net zoals een voormalig landbouwgebruik, van minder belang op de organisatie van de plantensoorten in de gemeenschappen. De soorten in deze gemeenschappen herschikten zich zodat de grotere planten de bovenhand kregen bij lichttoediening en opwarming, terwijl voorjaarsbloeiers standhielden in donkere condities (Hoofdstuk 6). Veranderingen in planthoogte van de gemeenschap kan je bijgevolg inschatten door rekening te houden met de algemene verschillen in planthoogte tussen kruidlaagsoorten. Om bladkenmerken op te schalen naar de gemeenschap is het echter wel nodig om met individualistische responsen rekening te houden, omdat verschillende groeicondities voor variatie in deze kenmerken kunnen zorgen die ook nog eens doorwegen op het gemeenschapsniveau.

Onze bevindingen impliceren dat subtiele verschillen in lichtbeschibkaarheid en luchttemperaturen relatief grote gemeenschapsveranderingen kunnen teweegbrengen op de korte termijn, en dit zowel binnen soorten als tussen soorten. Tegen onze verwachtingen in vonden we weinig bewijs voor effecten van N aanrijking en een voormalig landbouwgebruik op korte-termijn ontwikkeling van kruidlaaggemeenschappen in bossen. Het zou dus goed kunnen dat plantengroei op deze bodems niet gelimiteerd was door N en P beschikbaarheid, maar meer door lichtbeschikbaarheid en groeitemperaturen in de lente. De afwezigheid van effecten van nutrientenbeschikbaarheid op de korte termijn impliceert niet dat deze factoren weinig invloed hebben op de ontwikkeling van de kruidlaag. Het toont eerder de complementariteit aan van experimenteel onderzoek ten opzichte van lange-termijn vegetatieopnames in bossen, of modelleerwerk, waar zulke effecten wel geobserveerd worden. Meer tijd in het experiment zou dus kunnen onthullen of er verschillende gemeenschapsresponsen door deze veranderingen in het leefmilieu kunnen optreden in de toekomst.

De bevindingen die we hier presenteerden kunnen van toegevoegde waarde zijn voor verder onderzoek in de *global change ecology,* de tak van ecologie die zich toespitst op veranderingen in leefmilieu en hun effecten op de organisatie van organismen. Een belangrijke bevinding is de variatie in plantenkenmerken binnen soorten, die samen met de herschikking van soorten de respons van een gemeenschap ten opzichte van het veranderend leefmilieu bepaalt. Hier is het ook van belang om te erkennen dat subtiele verschillen in lichthoeveelheid de responsen in plantenkenmerken op gemeenschapsniveau kunnen bepalen, en zelfs kan doorwegen op die van andere veranderingen in het leefmilieu. Verder onderzoek in deze lijn kan de interacties tussen plant- en bodemorganismen bestuderen, en nagaan hoe dit het functioneren van het bos in de toekomst kan bepalen. Onze huidige bevindingen bieden ook perspectief voor een adaptief bosbeheer voor klimaatsverandering, door aan te tonen dat een relatief lage lichtbeschikbaarheid de sleutel is tot een succesvolle restoratie van kruidlaaggemeenschappen in bossen. Ecologisch onderzoek kan dus de tools leveren voor beleidsmakers die de intentie hebben om bossen te herstellen zodat effecten van klimaatsverandering en andere wijzigingen in het leefmilieu kunnen gekeerd worden. Deze beslissingen zouden het welzijn van mens en natuur ten goede komen, zeker nu het momentum is aangebroken dankzij de *United Nations Decade of Ecosystem Restoration* (2021 – 2030).

Image: Haben Blondeel

Abbreviations

Abbreviations

Chemical compounds and elements

Al	Aluminium
NH_4^+	Ammonium
NH_4NO_3	Ammoniumnitrate
C	Carbon
CH_4	methane
CO_2	carbon dioxide
K	Potassium
Mg	Magnesium
N_2O	nitrous oxide
Na	Sodium
NO_3^-	Nitrate
N	Nitrogen
P	Phosphorus
S	Sulphur

General terms

AM	Amiens region, France
ANOVA	Analysis of Variance
BC	Base Cations
BR	Bremen region, Germany
CCI	Colonisation Capacity Index
CH	Chimay region, Belgium
COM	Species combination
CV	Coefficient of Variation
CWM	Community weighted mean
D.F.	Denominator degrees of freedom
DEIMS - SDR	Dynamic Ecological Information Management System - Site and Dataset Registry
EMEP	European Monitoring and Evaluation Programme on air pollutioon
ERC	European Research Council
EST	Lääne Eesti region, Estonia
HRF	Hierarchical response framework
ICP s	International Cooperative Programme on Assessment and Monitoring of Air Pollution
INBO	Instituut voor Natuur en Bosonderzoek
IPBES	Intergouvernemental Panel on Biodiversity and Ecosystem Services
IPCC	Intergouvernemental Panel on Climate Change
ITV	Intraspecific trait variation
L	Light treatment
LOESS	Locally estimated scatterplot smoothing
LTER	Long-Term Ecological Research
LU	Land-use treatment
LUH	Land-use history
MAP	Mean annual precipitation (mm)
MAT	Mean annual temperature (°C)
MEA	Millennium Ecosystem Assessment
N	Nitrogen treatment
Ndfl%	Percentage N derived from label
NUTS	Nomenclature of Territorial Units for Statistics
OR	Orléans region, France
OTC	Open Top Chamber
PCA	Principal Component Analysis
PR	Prigitz region, Germany
PRC	Principal response curves
RDA	Redundancy Analysis
RGB	Red-Green-Blue
RR	Response Ratio
S.E.	Standard error
SEM	Structural equation model
SLA	Specific leaf area
SW	Skåne region, Southern Sweden
T	Temperature treatment
VL	Vlaanderen, Belgium
WRB	World Reference Base for Soil Nomenclature

Image: Sofie Verstraelen

Chapter 1

General Introduction

Plant biodiversity in a changing world

Terrestrial plants represent 80% of the living biomass on Earth (Bar-On, Phillips, & Milo, 2018) and are thus crucial to support ecosystem functioning in a changing world (Cardinale et al., 2012; Mori, Lertzman, & Gustafsson, 2016; Suding et al., 2008). Anthropogenic global change has been accelerating since the rise of the industrial revolution, and has launched a distinct geological time period often referred to as the Anthropocene (Steffen, Grinevald, Crutzen, & McNeill, 2011; Waters et al., 2016). The Anthropocene is characterised by alarming trends in anthropogenic activities which pressure the environment (Ripple et al., 2017) and the Earth's resilience as a whole (Figure 1.1.). Important global change drivers are land-use change leading to habitat loss and altered ecosystems (Fahrig, 2003; Newbold et al., 2015), disturbed biogeochemical cycles (Borer et al., 2019; Craine et al., 2018; Powers et al., 2019; Stevens, 2019), and emissions of greenhouse gases causing climate change (Santer et al., 2019). Together, these anthropogenic global changes can threaten plant biodiversity from the global down to the local scale (Gonzalez et al., 2016; McGill, Dornelas, Gotelli, & Magurran, 2015; Vellend et al., 2017).

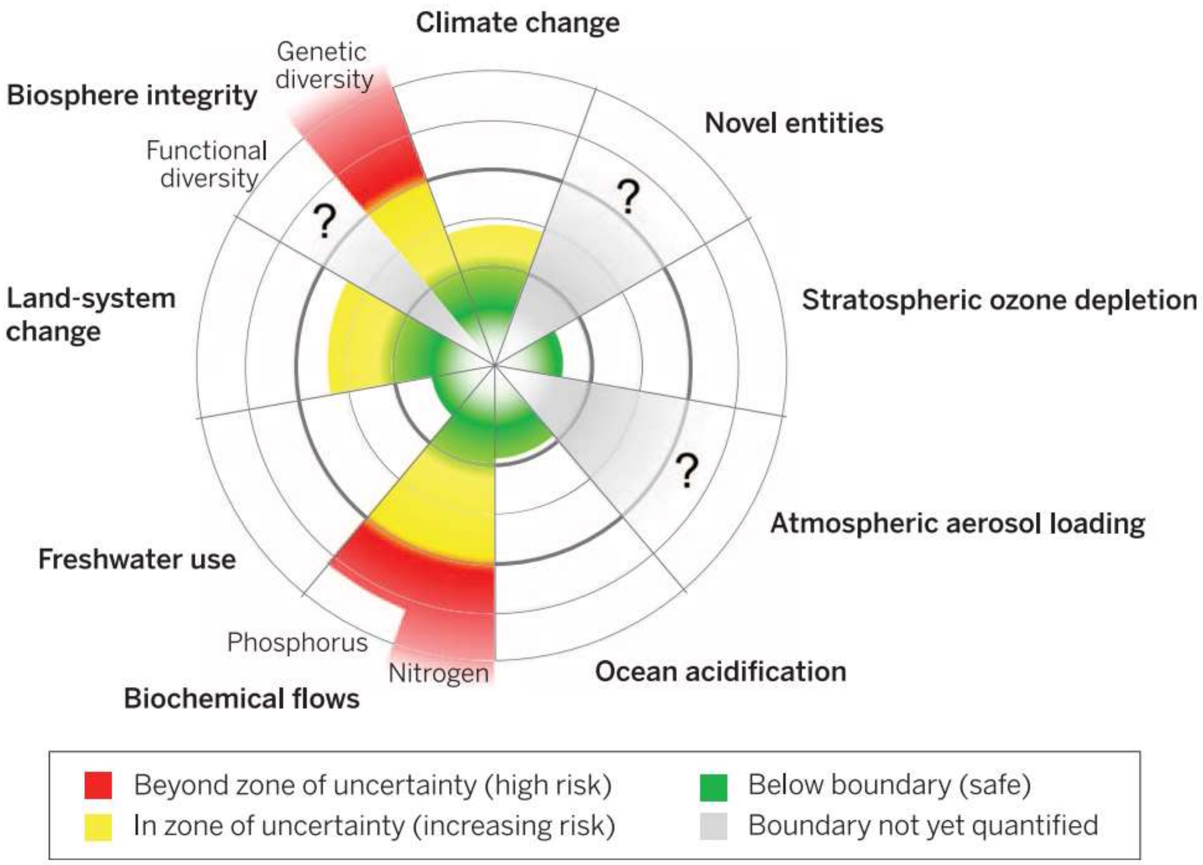


Figure 1.1. The "Planetary Boundaries" concept defines a safe operating space to guide humanity into a sustainable future (figure and concept by Steffen et al., 2015). Currently, biogeochemical flows and the biosphere are at the largest risk of irreversible change, followed by land-use change and climate change.

Contemporary global change, as outlined in the planetary boundaries concept (Steffen et al., 2015), needs to be further contextualised by important previous changes to improve our understanding of future ecosystem responses (Ogle et al., 2015). Important prior changes that influence contemporary ecosystem response to environmental change can be those of legacies from past land use (Bürgi, Östlund, & Mladenoff, 2017; Perring et al., 2016). Land-use legacies are any alterations in abiotic resources and conditions, and biotic patterns and processes, brought about by prior land use in terrestrial ecosystems (Bürgi et al., 2017; Foster et al., 2003; Hermy & Verheyen, 2007). Community processes of dispersal, selection, drift and speciation can then follow to steer communities and ecosystem functions further onto trajectories of change (Perring et al., 2016; Vellend, 2010). Furthermore, land-use legacies often enforce lagging ecosystem dynamics, so that ecosystems with an e.g. slower development have more potential to be influenced by their effects (Bürgi et al., 2017). Effects of land-use legacies are consequently well-documented in temperate forests, and particularly on community dynamics in the herbaceous understorey (Abadie et al., 2018; Brudvig, Grman, Habeck, Orrock, & Ledvina, 2013; Hermy & Verheyen, 2007; Newbold et al., 2015; Verheyen, Bossuyt, Hermy, & Tack, 1999).

Forest understoreys are thus a valuable system for studying legacy effects on plant communities in different global change contexts, as this PhD thesis presents. The forest understorey is here defined as the ground layer of forest vegetation with a high abundance of herbaceous species, which can represent up to 90% of the plant species richness in temperate forests (Gilliam, 2007; Večeřa et al., 2019).The understorey can be important to many ecosystem functions, including tree regeneration, primary production, evapotranspiration, supporting the forest food web, and nutrient cycling (Landuyt et al., 2019). Furthermore, these functionally rich communities can have an important "early-warning" potential for forest responses to global environmental change (Verheyen et al., 2017). The importance of the herbaceous understorey to the forest functioning is driven by the functional traits of the understorey species (Landuyt et al., 2019; Landuyt, Maes et al. (in revision); Gilliam, 2007). Functional traits are morphological, physiological or phenological features of organisms that characterise species' ecological strategies (Funk et al., 2017; Laughlin, Leppert, Moore, & Sieg, 2010; Violle et al., 2007) and are tightly linked to an organisms' environment (Bruelheide et al., 2018; Clark, 2016). Traits can thus respond to changes in the environment and in turn affect the functioning of the ecosystem (Funk et al., 2017; Suding et al., 2008), so that environmental change effects on plant communities may be measured by change in functional traits (McGill, Enquist, Weiher, & Westoby, 2006). In the coming sections and research chapters, we investigate the altered resources and conditions that common global change drivers can engender, before exploring whether individualistic plant species' trait responses scale up to a community-level change in traits.

Land-use legacies drive temperate forest understorey development

Land-use legacies alter resources and conditions in temperate forests

A former arable land-use can spawn persistent legacy effects in temperate forests (Cramer, Hobbs, & Standish, 2008), more than abandoned heathland (Bizzari, Collins, Brudvig, & Damschen, 2015; Grossmann & Mladenoff, 2008) or former pastures (Compton & Boone, 2000). This stronger agricultural legacy is a consequence of prior fertilisation practices (Cramer et al., 2008; Macdonald, Bennett, & Taranu, 2012; Rowe et al., 2016), previous ploughing (Flinn, Vellend, & Marks, 2005) and even the past use of pesticides (Rasmussen et al., 2015). Together, these previous alterations of the pedosphere can cause an enrichment in nutrients for centuries (Verheyen et al., 1999) up to millennia (Dupouey, Dambrine, Laffite, & Moares, 2002). The influence of agricultural legacy on soil chemistry can however be variable, but generally involves a lower carbon (C) to nitrogen (N) ratio, higher pH and larger availability of phosphorus (P) in particular (Compton & Boone, 2000; Leuschner, Wulf, Bäuchler, & Hertel, 2014). This variability in agricultural soil legacy arises at a regional and continental level and depends on specific management practices that were followed during the agricultural period (Brudvig et al., 2013; Macdonald et al., 2012; McLauchlan, 2006). It is thus possible for numerous accounts of agricultural legacies in forest soil properties to be contrasting and site specific when compared to each other (Baeten, 2010). Chapter 2 in this thesis aims to reconcile these contrasting legacies, by considering gradients of inherent soil characteristics, regional P management and N deposition.

Land-use legacy can also affect the soil biota in forests, alongside the alteration of the abiotic soil environment (Foster et al., 2003). For instance, the colonisation of mycorrhizal fungi can be impeded for decades in forests with a recent agricultural land-use history (Boeraeve et al., 2018; Fichtner, von Oheimb, Härdtle, Wilken, & Gutknecht, 2014). The lacking presence of these symbionts may inhibit the establishment of colonising plants, which can determine understorey composition for decades (Jo, Potter, Domke, & Fei, 2018).

Land-use legacies drive the composition of the understorey for centuries

The forest land-use history drives the composition of understorey communities for decades to centuries with an important role of dispersal limitation (Brunet, De Frenne, Holmström, & Mayr, 2012a; De Frenne, Baeten, et al., 2011; Hermy & Verheyen, 2007; Naaf & Kolk, 2015; Naaf & Wulf, 2012). Land that has been continuously forested for centuries is regarded as “ancient forest” (Hermy & Verheyen, 2007). These ancient forests can be characterised by an understorey that is composed of slow colonising herbaceous species (Figure 1.2A) with poor dispersal (Verheyen, Honnay, Motzkin, Hermy, & Foster, 2003). The inadequate dispersal of this typical woodland flora are consistent predictors for understorey recovery when spatial isolation and time since agricultural abandonment are taken into account (Van Der Veken, Rogister, Verheyen, Hermy, & Nathan, 2007; Verheyen & Hermy, 2001a, 2001b).

Figure 1.2. Spring in the Aelmoeseneie forest (Gontrode, Belgium). Ancient forests stands in this woodland can be dominated by the spring ephemeral *Anemone nemorosa* (A). Adjacent stands with a post-agricultural land-use history have a markedly different understorey composition, where the nitrophile *Urtica dioica* dominates (B).

Environmental limitation can be the second filter, preceded by dispersal limitation, that influences the recovery of herbaceous communities in forests with a recent agricultural history (Flinn & Vellend, 2005; Hermy & Verheyen, 2007). Reduced performance of adult individuals in such “post-agricultural” forests would be an indicator of environmental limitation in slow colonising understorey plants (Baeten, Hermy, & Verheyen, 2009). Controlled plant introduction experiments in forests with contrasting land-use history have revealed that, indeed, nutrient enrichment and changes in light availability can inhibit the presence and abundance of typical understorey species after 20 years of initial recruitment (Baeten & Verheyen, 2017). This inhibition is followed by competitive exclusion, where prior incumbents are capitalising on the resources and can exclude new arrivals from coexistence (Bhaskar, Dawson, & Balvanera, 2014; DeMalach, Zaady, Weiner, & Kadmon, 2016; Kraft, Godoy, &

Levine, 2015). Understoreys in post-agricultural forest can thus be dominated for decades by fast colonising species (Verheyen et al., 2003; De Frenne et al., 2011, see Figure 1.2B) which inhibits the colonisation of slow colonisers (Kolk, Naaf, & Wulf, 2016; Naaf & Kolk, 2015).

Past land use, and the associated community processes of dispersal limitation, environmental limitation and competitive exclusion, can have an imprint on understorey community composition for prolonged times (Newbold et al., 2015). The development of forest understoreys is, however, not entirely determined by land-use legacies alone (Perring et al., 2016). Further modulation of the development trajectories that past land use has engendered can depend on chronic global change such as N deposition and climate change, or on pulse disturbance caused by the management of ecosystems (Perring, Bernhardt-Römermann, et al., 2018; Perring et al., 2016; Perring, Diekmann, et al., 2018; Smith, Knapp, & Collins, 2009).

N deposition causes a eutrophication signal in the understorey

N deposition is an environmental change driver that varies regionally and originates from intensive agriculture and burning of fossil fuels (Bobbink et al., 2010). N deposition is consequently largest in densely populated areas and in regions with many intensive livestock farms, and decreases in colder northern latitudes (De Frenne et al., 2013, see Figure 1.3). N deposition increases reactive N in the environment (Stevens, 2019) via dry or wet depositions, i.e. dust particles and precipitation respectively (Erisman & Draaijers, 2003). The deposition of N becomes available for plant uptake as water soluble ammonium (NH_4^+) or nitrate (NO_3^-) ions, which can be taken up directly or after microbial N transformations such as nitrification (Lukac & Godbold, 2011). Reactive N in the environment causes acidification and eutrophication which affects ecosystem composition and functions (De Schrijver et al., 2011) at an ecosystem-specific critical load (Bobbink et al., 2015). Critical loads for different deciduous forest types range from 10 to 20 kg N ha^{-1} y^{-1} (Bobbink et al., 2010, 2015; Simkin et al., 2016). Adverse effects from acidification can follow as the exceedance of critical loads potentially leaches N and base cations out of the environment, so that phytotoxic aluminium compounds can become bio-available at a pH below 4.2 (Bobbink et al., 2010, 2015; Lukac & Godbold, 2011). More N inputs can saturate the environment which leads to eutrophication (Bobbink et al., 2010). At this saturation point, there is no further additional impact of N because other resources have now become limiting for growth (Bobbink et al., 2010; Brummer & Khanna, 2008; Craine et al., 2018; Zhou, Guo, Zhang, & Du, 2018).

Acidification and eutrophication driven by cumulative N deposition can drive species richness changes in understorey communities when forests are N saturated (De Schrijver et al., 2011;

Rainey, Nadelhoffer, Silver, & Martha, 2018; Soons et al., 2017; Walter, Adams, Gilliam, & Peterjohn, 2017). Richness changes have been attributed to the dominating presence of nutrient demanding herb species (Gilliam et al., 2016). This eutrophication signal in the understorey is general across wide geographic gradients (van Dobben & de Vries, 2017; Verheyen et al., 2012), but with species richness changes usually only following when light availability in the forest also increases (Perring, Diekmann, et al., 2018; Simkin et al., 2016; Walter et al., 2016). The accumulated N over time could thus present an “N time bomb” which could explode when the forest canopy opens, leading to the sudden abundance increase of a few nutrient demanding species (Verheyen et al., 2012). Consequently, even with policies having enforced declining N depositions during the past 20 years (Erisman, Grennfelt, & Sutton, 2003; Verstraeten et al., 2012), recovery in European temperate forest understoreys did not yet follow where past depositions were exceedingly high (Dirnböck et al., 2018; Schmitz et al., 2019).

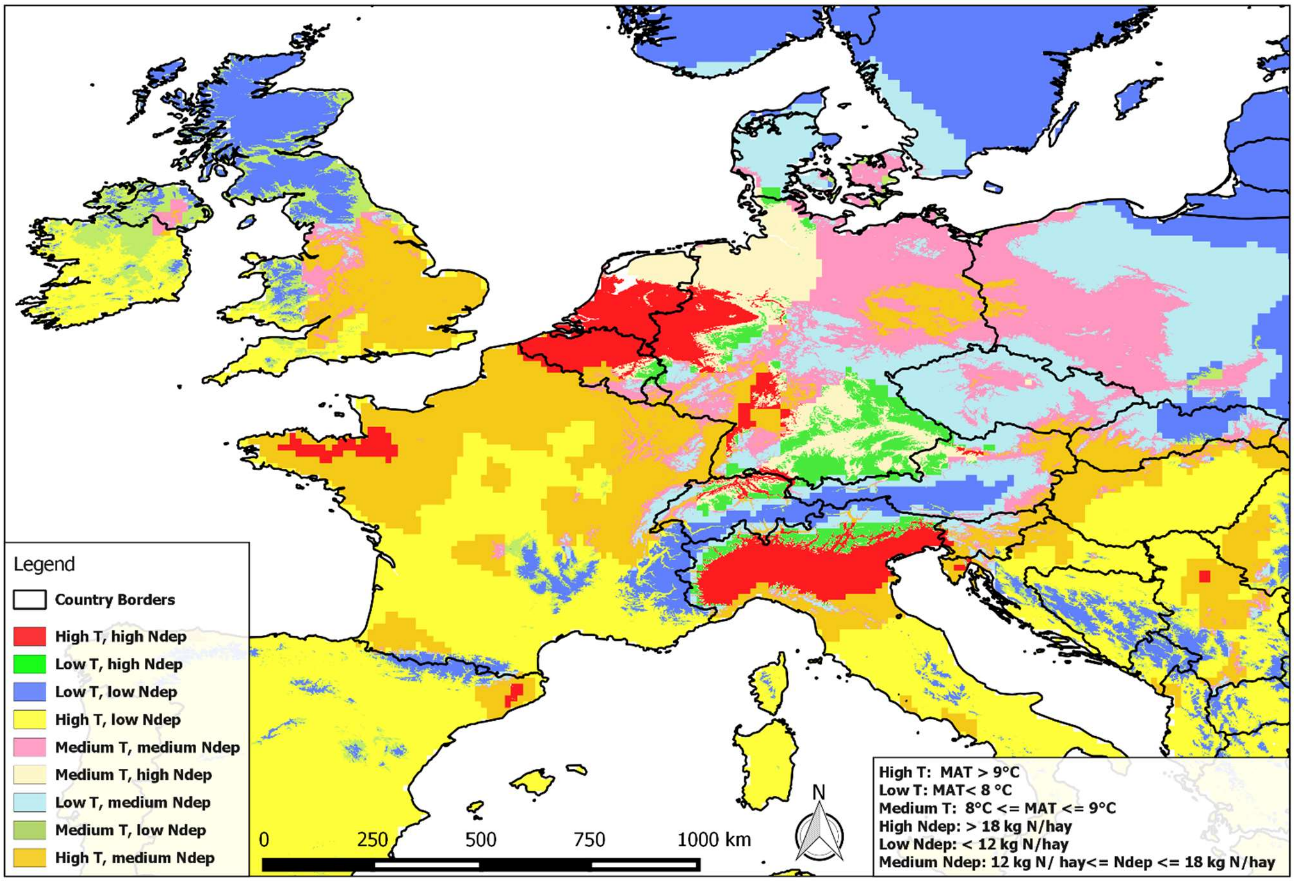


Figure 1.3. N deposition varies in Europe by region, with densely populated and intensively farmed regions showing higher N deposition. In cold northern latitudes (Denmark and further north), regions become sparsely populated and less intensively farmed, so that both N deposition and mean annual temperature (MAT) decrease. The data to create this image was drawn from the WorldClim climate database (1950 – 2000, www.worldclim.org) and the EMEP air pollution database (50 x 50 km^2) interpolated modelled N deposition for the year 2000, www.emep.int).

Climate change at alarming rates

Climate change is a global change driver that originates from anthropogenic emissions of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) which cause a greenhouse warming effect (IPCC, 2014). These greenhouse gasses have already caused a global increase in mean surface temperature of approximately 1°C (Santer et al., 2019), with warming likely to reach 1.5 °C between 2030 and 2052 if emissions continue at current rates (IPCC, 2018; Power & Delage, 2019). The projected climate change can increase extreme weather events and precipitation patterns across the globe (IPCC, 2018). The rise in global CO_2 in the troposphere has recently reached the unprecedented benchmark of 400 ppm, the highest level for over 800,000 years (Betts, Jones, Knight, Keeling, & Kennedy, 2016; Dyez, Hönisch, & Schmidt, 2018; Showstack, 2013). This press elevation in CO_2 can alter functioning of terrestrial ecosystems (Sardans et al., 2017; Yin, 2002). Vegetation around the globe is experiencing enhanced peak growth due to CO_2 fertilisation (Huang et al., 2018; Zhu et al., 2016), which can cause limitations in other resources such as N (Craine et al., 2018).

Warming, rather than changing precipitation or increasing CO_2, is likely to be the foremost aspect of climate change to affect herbaceous understoreys in northern temperate forests (Rollinson & Kaye, 2012). Mean annual precipitation in northern temperate regions is not expected to change much in future climate scenarios (IPCC, 2014), while effects of extreme precipitation variability on plant communities remains unclear (Reyer et al., 2013). Even then, herbaceous plants seem less affected by changing precipitation (Rollinson & Kaye, 2012; Rollinson, Kaye, & Leites, 2012) or increased CO_2 (Pan, Birdsey, Hom, & McCullough, 2009; Sardans et al., 2017) than trees due to a lesser importance of woody biomass in ontogeny of herbaceous plants. On the long term, herbaceous understorey species can orient their ranges towards the poles in order to chase suitable growing climates (Skov & Svenning, 2004). In addition, herbaceous understorey communities also respond to short term warming, as communities reorder to an increasing abundance of warmth-adapted species when light availability is high (De Frenne et al., 2015; Helsen et al., 2017; Hoeppner & Dukes, 2012).

Forest management change influences gap dynamics

Forest management is an important driver of resources and conditions in forest as it affects the light availability, the forest microclimate and canopy composition. The canopy composition in northern temperate forest is largely driven by choices of forest managers to deliver certain ecosystems services such as production (Baeten et al., 2019). Knowingly or unknowingly, these tree species choices can drastically alter the soil nutrient availability via the litter quality of those trees (Cools, Vesterdal, De Vos, Vanguelova, & Hansen, 2014; Maes et al., 2019). Forest managers can additionally choose to influence the light avaibility and the stand structure in the forest (Pretzsch, Biber, Schütze, Uhl, & Rötzer, 2014; Sercu et al., 2017). Management practices such as regeneration cuts or thinning remove direct competitors of future crop trees in forest stands. These interventions serve to increase growing space for these future crop trees but also bring more light to the forest floor (Hedwall, Strengbom, & Nordin, 2013). This increasing light availability can alter soil microbial communities and enhance their activity to raise availability in N (Ma et al., 2018; Ni et al., 2018). Canopy gaps also receive more wet N deposition, along with the raised light availability and warmer temperatures due to the loss of the microclimate buffering canopy trees (De Frenne et al., 2019; Zellweger et al., 2019, see Figure 1.4).

Figure 1.4. Panoramic view of a two-year old forest gap. The image was taken in the Aelmoeseneie forest (Belgium). Notice the large abundance of the fast growing species *Pteridium aquilinum* in the understorey.

The causality in understorey responses to gap conditions is difficult to reveal in observational field studies, as light availability in forest gaps is entangled with sub canopy warming (Valladares, Laanisto, Niinemets, & Zavala, 2016) and even N availability (De Frenne et al., 2018; Ni et al., 2018). Short–term experimental data show that it is increasing light availability that can accelerate herbaceous community responses to warming (De Frenne et al., 2015) and eutrophication (Hautier, Niklaus, & Hector, 2009), by shifting the community towards a dominance of warmth-adapted and nutrient demanding species (DeMalach, Zaady, & Kadmon, 2017; Walter et al., 2016). Long-term resurveys in temperate European forest have shown that light avaibility at the forest floor generally decreased due to declining forest management intensity (McGrath et al., 2015), but with regional increases where pest outbreaks have led to increased tree mortality (Senf et al., 2018). This general decrease in light availability has steered the understorey composition towards shade tolerant species decades after the canopy disturbances (Mölder, Streit, & Schmidt, 2014; Verheyen et al., 2012; Verstraeten et al., 2013). Likewise, management transitions from temporarily open forests (such as a coppice with standards system) to densely packed high forests can yield similar “darkening” signals in community traits of the understorey (Perring, Bernhardt-Römermann, et al., 2018). Light avaibility can thus play an important role in the development of understorey community trajectories in different global change contexts (Depauw et al., 2019).

Interactive effects of land-use legacies and global change on plants

Land-use legacies could modulate trajectories of understorey community responses to global environmental change, but observational evidence for such interactions is scarce (Perring et al., 2016). Understanding the isolated effects of environmental change drivers on understorey plants in ancient vs. post-agricultural forests is challenging because dispersal limitation naturally determines functional trait distributions in those forests (Brunet, De Frenne, et al., 2012a; De Frenne, Baeten, et al., 2011). However, controlled plant introduction experiments could tease apart whether plant responses to N enrichment, climate change and light differ with the forest land-use history (Luo et al., 2011; Verheyen et al., 2017). In a recent study, Barker et al. (2019) found that understorey establishment was not limited in post-agricultural forest compared to a controlled introduction in ancient forests. Furthermore, past land use seemed to modulate responses to canopy disturbances, as more species were only established in post-agricultural forests with high canopy disturbance. In a longer term experiment, Baeten & Verheyen (2017) have found that establishment of two contrasting understorey species was more divergent in post-agricultural forest than ancient forest after canopy disturbance. The abundance of the slow colonising species *Primula elatior* had decreased in post-agricultural forest compared to ancient forest, while the fast colonising *Geum urbanum* had not changed in abundance. These canopy disturbances had increased light availability, N deposition and subcanopy temperatures over the course of 20 years, which had put both species on new and unexpected development trajectories.

Land-use legacies, N enrichment, climate warming and light availability together alter resources and conditions in forests, and could spawn complex interactive effects on the development of forest understoreys (Landuyt et al., 2018; Verheyen et al., 2017). These potential interactive effects can be understood in the light of multiple resource limitation theory and its repurcussions for species coexistence (Dutta, Kooi, & Feudel, 2014; but see De Laender, 2018). When environmental change alters limiting resource or conditions, individual plants can take opportunities and exploit previously untapped resources in the community. Species that respond too slowly to these changes risk being competitevly excluded. In general, resource limitation theories predict that increasing light availability can modulate the herb layer development trajectory in sites where N and P are less limiting to plant growth (Grime, 2001; Jabot & Pottier, 2012; Tilman, 1988). Common examples are that warmth-adapted species can increase in abundance in bright and warm forest gap conditions, with light as the accelerating driver for this warming response (De Frenne et al., 2015). Likewise, the dominance of nutrient demanding species can increase under enrichment of N and P when light availability is high (Fraterrigo, Pearson, & Turner, 2009; Gilliam et al., 2016; Holmes & Matlack, 2017; Siefert & Ritchie, 2016; Walter et al., 2016). While these examples were established on the community level, long-time evidence suggests that plant responses after changing resources and conditions scale from individuals up to the community (Chapin & Shaver, 1985). We designed and performed an *in situ* mesocosm experiment to disentangle these environmental change drivers on development of forest herb individuals and communities (Chapter 3).

Plant responses to global change: from individual to community

An important way to understand and scale plant functional responses to environmental change from individuals to the community is in the light of the “response and effect trait framework” (Suding et al., 2008). This framework first describes that responses of plant communities to environmental change can be better predicted from species traits, rather than species identity (Funk et al., 2017; Lavorel & Garnier, 2002; Suding et al., 2008). Second, altered functional traits in plant communities (e.g. leaf traits) can have important effects on key ecosystem functions (Funk et al., 2017; Suding et al., 2008), for instance on nutrient cycling (Cornwell et al., 2008; Firn et al., 2019; Hobbie, 2015). Ecologists should thus consider studying traits for being responsive to environmental change (“response” traits), and/or whether change in traits could affect key ecosystem functions (“effect” traits).

Individualistic plant responses to global change

Patterns in plant species growth and functional trait responses to warming (Hollister, Webber, & Bay, 2005), light availability (Rozendaal, Hurtado, & Poorter, 2006) and nutrient enrichment (Fynn & O’Connor, 2005) have long been shown to be dependent on the species. Such individualistic species responses suggest different limiting factors for plant species that naturally occur in the same communities (Chapin & Shaver, 1985). These individualistic plant responses are controlled by a species’ ecological strategy (Garnier, Navas, & Grigulis, 2016b; Jagodziński, Dyderski, Rawlik, & Katna, 2016; Mao, Felton, & Zhang, 2017; Poorter et al., 2009), which can be defined via traits related to colonisation ability, life-history, and leaf and whole plant economics (Díaz et al., 2016; Garnier, Navas, et al., 2016b; Reich, 2014).

A classical scheme to describe ecological strategies is to account for trade-offs and variation in leaf-height-seed traits, to cover plant processes of growth, resource acquisition and reproduction (Díaz et al., 2016; Laughlin et al., 2010; Reich, 2014; Westoby, 1998). Functional leaf traits follow a “leaf economics spectrum”, which represents trade-offs in leaf construction (Giancarlo, Marouane, & Bill, 2010; Messier, McGill, Enquist, & Lechowicz, 2017; Wright et al., 2004). These trade-offs allow to either specialise in resource acquisition, with high leaf area to leaf mass ratio (specific leaf area, SLA) and high N concentration, or resource conservation with low SLA and leaf N concentration. Similar trade-offs occur in plant height (Beckman, Bullock, & Salguero-Gómez, 2018; Gibert et al., 2016; Moles et al., 2009; Thomson, Moles, Auld, & Kingsford, 2011), where high relative growth rates, low lifespan and tall size correlate with resource acquisition in forest herbs (Verheyen, Honnay, et al., 2003). Variation in seed traits (e.g. mass) can be important to distinguish between plant species and large taxonomical groups (Díaz et al., 2016; Jiang, Xun, Cai, & Jin, 2018; Moles & Westoby, 2004; Westoby, Falster, Moles, Vesk, & Wright, 2002). Within herbaceous species, and particularly in forest understoreys, seed trait responses can be ambiguous because bud banks can be more important for the proliferation of plants (Garnier, Navas, & Grigulis, 2016a; Klimešová, Tackenberg, & Herben, 2016; Ott, Klimešová, & Hartnett, 2019). We therefore chose to focus on leaf traits (i.e. SLA and leaf N content per dry mass) and plant height as the focal functional traits throughout this study, as did Myers-Smith,et al. (2019) in recent work in the tundra biome (Figure 1.5).

There can be large interspecific differences in mean trait values of forest herbs depending on a species’ ecological strategy (Verheyen, Honnay, et al., 2003). Herbaceous species that are confined to ancient forests are typically small-statured slow colonisers that can photosynthesise in mature forests, either as spring-flowering geophytes (Mabry, Gerken, & Thompson, 2008; Rothstein, 2000; Tessier & Raynal, 2003) or as shade tolerant plants that grow under fully-developed tree canopies (Valladares & Niinemets, 2008). These perennial ancient forest herbs consequently invest more nutrients into roots, bulbs, suckers or large seeds for proliferation (Verheyen, Honnay, et al., 2003), but at a cost of slow dispersal (Klimešová et al., 2016).

The ability of forest herbs to colonise post-agricultural forests correlates positively with relative growth rate and aging quickly (Verheyen, Honnay, et al., 2003), both plant characteristics indicative of a “fast life-history” (Adler et al., 2014; Beckman et al., 2018). Such fast colonisers are tall with light seed which allows further dispersal than species that are typical of ancient forest (Beckman et al., 2018; Thomson, Moles, Auld, & Kingsford, 2011; Verheyen, Honnay, et al., 2003). Fast colonising forest herbs additionally have a high SLA and high leaf N content (Verheyen, Honnay, et al., 2003) which is a leaf construction that favours resource acquisition over resource conservation (Adler et al., 2014; Díaz et al., 2016; Reich, 2014; Wright et al., 2004).

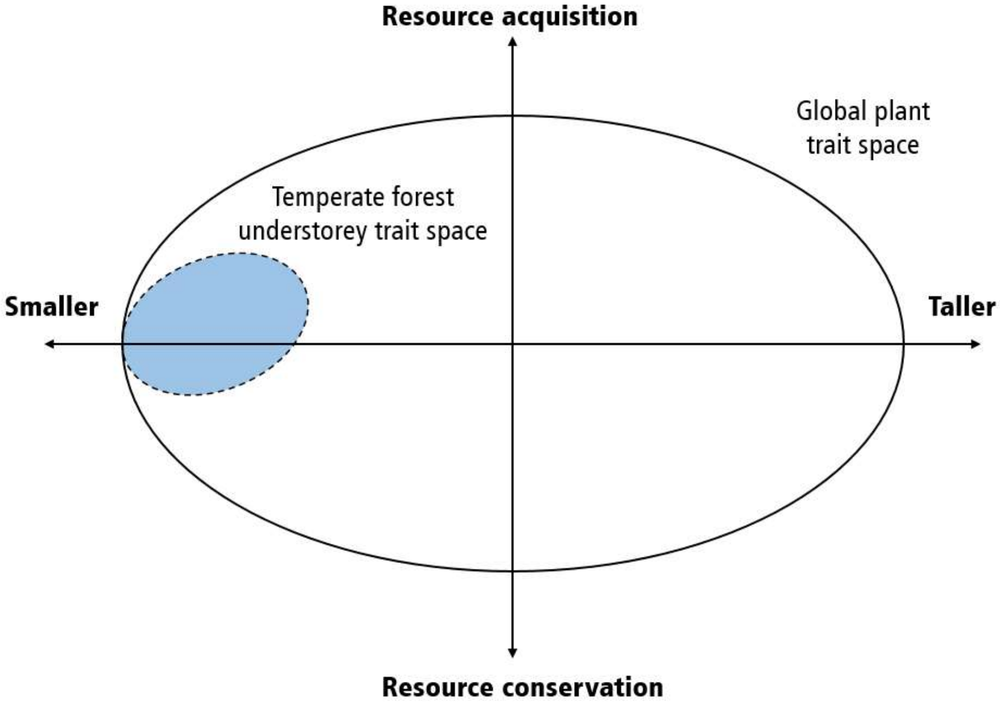


Figure 1.5. Plant height and resource acquisition traits are key plant functional traits across the globe. This figure is not based on a formal analysis, but merely an adaptation from a conceptual graphical representation of the global plant trait space with indepent axes of plant height and resource acquisition traits (see Myers-Smith et al. (2019) for this scheme in the tundra biome). The trait space in temperate forest understoreys is merely a small subset of the global trait space, which is drawn here as one nested subset oval within another. Resource acquisition (i.e. leaf economic traits) and plant height correlate positively in forest herbs (Verheyen, Honnay, et al., 2003), so that this subset oval has a slanted shape.

Patterns in within and between species variation in these functional traits can be important to understand community-level trait responses over time (Albert, Grassein, Schurr, Vieilledent, & Violle, 2011; Albert et al., 2010; Moran, Hartig, & Bell, 2016). Chapter 4 aims to assess whether within-species trait variation due to altered resources and conditions depends on ecological strategies.

The functional signature of forest understoreys can influence many ecosystem functions, including tree regeneration, primary production, evapotranspiration, and nutrient cycling (Landuyt et al., 2019; Landuyt, Maes et al. (in revision); Gilliam, 2007). Nutrient cycling via the understorey can be of particular importance in forest ecosystems, despite the understorey representing about half of the foliar biomass in comparison with the overstorey trees (Landuyt, De Lombaerde et al., 2019); Landuyt, Maes et al. (in revision); Gilliam, 2007). Foliage of herbaceous understorey plants have, however, generally higher concentrations of key nutrients (N, P, K, Ca, Mg) than the foliage of trees (Gilliam, 2014; Refsland & Fraterrigo, 2017; Welch, Belmont, & Randolph, 2007). Herbaceous species have a higher nutrient assimilation efficiency than canopy trees (Buchmann, Gebauer, & Schulze, 2004) and root in shallower nutrient rich topsoil layers (Jobbágy & Jackson, 2001). Moreover, more than woody species, herbaceous species tend to position themselves along the leaf economics spectrum towards resource acquisitive leaves with a high SLA, high N concentration and low leaf longevity (Díaz et al., 2016). Trees, and all other organisms that feed on soil N, can be promoted by this higher quality litter in early spring (Mabry et al., 2008; Rothstein, 2000). Soil N could otherwise have leached from the system if the herbaceous understorey was not there to temporarily lock nutrients in spring biomass ("vernal dam hypothesis", Eickmeier & Schussler, 1993; Tessier & Raynal, 2003). Foliar N is thus a key trait that links significance of the understorey to N cycling (Jagodziński et al., 2016; Tessier & Raynal, 2003).

Environmental change could affect foliar N of understorery communities due to physiological responses of individuals (“direct effects”) or via altered community properties in response to environmental change (“indirect effects”, see Manning et al., 2006). A common direct response of individual plants to higher light availability and climate change is a dilution of leaf N due to increases in plant size and carbon acquistioin rates (Jarrell & Beverly, 1981; Sardans et al., 2017; Yin, 2002). Contrastingly, a direct increase in leaf N can occur in response to N enrichment from deposition (Farrer & Suding, 2016; Fraterrigo et al., 2009) or legacies of prior agriculture (Baeten et al., 2011; Siefert & Ritchie, 2016). Indirect effects can occur as nutrient enrichment in warm and bright conditions could steer the community towards dominance of species with innate high tissue N (Gilliam et al., 2016; Walter et al., 2017) or simply raise community productivity (Baeten et al., 2011; Jagodziński et al., 2016). In Chapter 5, we explore the direct and indirect effects of multiple environmental changes on foliar N of understorey species, and how this links to resource acquisition strategies.

Scaling up individual plant responses to the community

The “hierarchical response framework” (HRF) introduces a framework to scale-up individualistic plant responses into a progressive plant community response (Smith et al., 2009). Plant communities first exhibit individualistic physiological responses to changing resources and conditions (Figure 1.6A, e.g. Chapin & Shaver, 1985). These individualistic responses are then followed by the reordering of species within communities, where some species in the community decrease in abundance at the expense of more fitted incumbent species (Figure 1.6B, e.g. Anderson et al., 2011). After a longer time period, resources and conditions can have changed considerably in the ecosystem so that more fitting species from outside the local species pool can invade (Figure 1.6C, e.g. Alexander et al., 2015). Likewise, incumbent species that now suffer from the changing resources and conditions can go extinct from the local species pool as “loser” species (Anderson et al., 2011; Li & Waller, 2015; Naaf & Wulf, 2011; Smith et al., 2009). Ecosystems dominated by very long-lived species with slow turnover rates in biomass, such as trees in forests, may appear to be resistant over time to such changes. Such communities could experience long lags in abundance changes, with the community response dominated by individualistic species responses (Figure 1.6D). Conversely, communities that are prone to invasion from exotic species may bypass the pathway where species in communities gradually reorder (Figure 1.6E). These final two exceptions to the HRF are expected to be of minor importance to the dynamics in our experimental study, because our experiment only includes non-invasive herbaceous species.

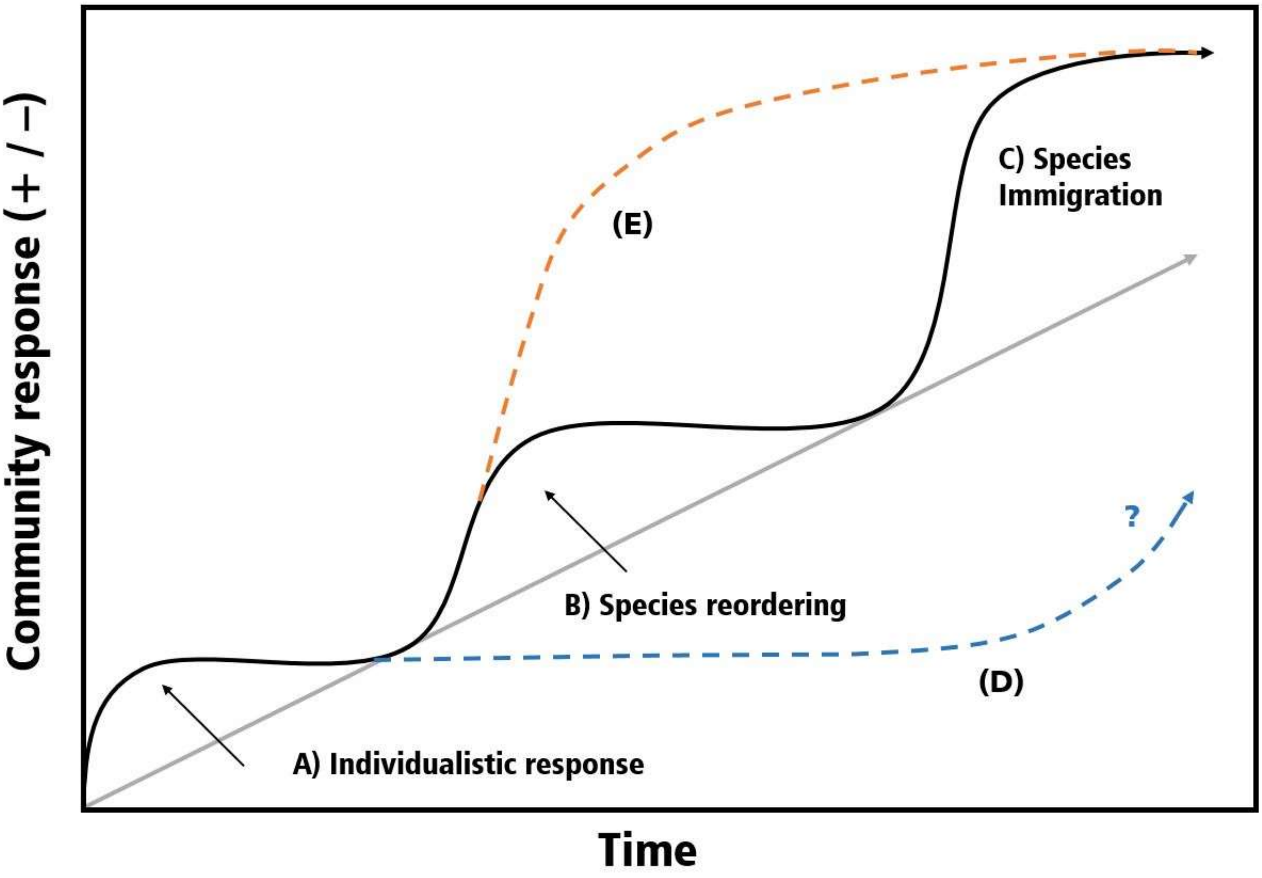


Figure 1.6. The Hierarchical Response Framework (HRF) describes a progressive, hierarchical and non-linear community response over time. This figure is adapted from Smith et al. (2009). Initial community responses are driven by relatively rapid individualistic (physiological) responses to changing resources and conditions (A). Larger shifts in the community response can be expected when species reorder within the community (B), as a result of some species being favoured by the changing environment at the expense of others. Longer periods are required for new immigrants to enter the community (C), which can lag due to dispersal limitation in potential better suited immigrants. The loss of species from the regional species pool (i.e. local extinctions) occurs within this stage of community response. Communities comprising of species that can only change very slowly in abundance (such as trees) may show a prongoled response dominated by individuals (D). Finally, invasive exotic species may bypass a gradual community reordering over time (E). These final two pathways are of minor importance to our experiment, which only includes non-invasive native herbaceous species.

This sequential community response over time can be estimated by assessing change in functional trait distributions of communities (McGill et al., 2006). Functional traits vary between species with different ecological strategies (Díaz et al., 2016; Garnier, Navas, et al., 2016a; Reich, 2014), but also within species due to local adaptation or plasticity in expression depending on the growing environment (Lajoie & Vellend, 2015, 2018; Valladares, Sanzecg-Gomez, & Zavala, 2006). This intraspecific trait variation (ITV) can have important contributions to community trait change in addition to species reordering (Albert et al., 2011, 2010; Bolnick et al., 2011; Siefert, 2012a). Species reordering and ITV can thus jointly influence community effects on long-term ecosystem functioning (Guittar, Goldberg, Klanderud, Telford, & Vandvik, 2016; Harte, Saleska, & Levy, 2015; Suding et al., 2008).

Chapter 6 aims to scale up individualistic trait responses to trait change in the community over time, in order to understand contrasting understorey development trajectories in different global change contexts.

Research gap: disentangling multiple global change drivers

Different methods can improve our understanding of how multiple global change drivers can affect plant communities over time (Luo et al. 2011). “Space-for-time” approaches along large environmental gradients can yield general insights in trait-environment relationships but rely on too many assumptions to predict community change over time (Damgaard, 2019). An alternative approach is to combine vegetation resurveys across a large spatial gradient (Verheyen et al., 2017). Such surveys are performed in (semi-) permanent plots, on at least two occasions with several decades between the surveys (Verheyen et al., 2017; Waller, Amatangelo, Johnson, & Rogers, 2012). These resurveys can provide robust estimates of species abundance changes over time in response to local and global environmental changes, despite some variation in relocation of those plots (Kopecky & Macek, 2015; Verheyen et al., 2018). These combined resurveys do, however, lack some degree of orthogonality, due to spatial confounding in environmental change drivers such as N deposition and rates of warming with e.g. latitude (De Frenne et al., 2013, see also Figure 1.3). Larger orthogonality between drivers can however be achieved in experimental research. Full-factorial treatments of several environmental change drivers in a multi-regional setting could then increase the generality of inference (Verheyen et al., 2017).

Multi-factor experiments that disentangle combined effects of light availability, warming and N enrichment have provided valuable insights to community ecology in the past decades (Bradford, Wood, Maestre, Reynolds, & Warren, 2012; Chapin & Shaver, 1985; De Frenne et al., 2015; Eickmeier & Schussler, 1993; Hautier et al., 2009; Manning et al., 2006; Walter et al., 2017). More recently, several frameworks have suggested that such orthogonal approaches are a necessary addition to observatory global change research (Luo et al., 2011; Verheyen et al., 2017), especially if researchers acknowledge the role of past land-use in modulating community trajectories (Perring et al., 2016). Experimental research that controls for land-use legacy effects on herbaceous communities has until now generally only included the role of light availability (Baeten, Vanhellemont, De Frenne, De Schrijver, et al., 2010; Hahn & Orrock, 2016), nutrient enrichment (Fraterrigo et al., 2009; Siefert & Ritchie, 2016) or warming (Hoeppner & Dukes, 2012; Walter et al., 2013) separately. This PhD thesis presents a full-factorial global change experiment to disentangle these drivers of herbaceous community development while accounting for past land use across multiple European regions.

Thesis objectives and outline

Objectives

The empirical ecological research presented in this PhD builds upon prior work of countless vegetation ecologists that studied herbaceous communities in temperate forests before (Szabó, 2015). This thesis sets out to project that research tradition into the future by studying forest responses of understorey development to global change. This predictive viewpoint is a central tenet of the PASTFORWARD project (www.pastforward.ugent.be, ERC Consolidator Grant 614839). PASTFORWARD is the overarching research project that provided the basis for the work here. This project combines methods of vegetation resurveys, mechanistic modelling and experimental work to understand understorey responses to global change while acknowledging the role of past land use. The research presented in this thesis accounts for the experimental work package of PASTFORWARD.

The main objectives of this thesis are to disentangle effects of light availability, N enrichment, warming and agricultural land-use history on development of forest understorey communities. We overcame dispersal limitation by planting slow and fast colonisers on soils from both ancient and post-agricultural forests, collected in eight regions across Europe. This planting scheme separates the dispersal limitation in species from the agricultural legacy, so that we can focus on the biogeochemical aspect of agricultural land-use legacy effects. We applied experimental treatments of warming, N addition and enhanced light availability to these “mesocosms” in a full-factorial approach. This full-factorial approach disentangles these common environmental drivers in temperate forest. The experimental set-up is used to investigate the altered resources and conditions that common global change drivers can engender in temperate forests, before exploring individualistic plant responses that could scale up to a community trait response (Figure 1.7).

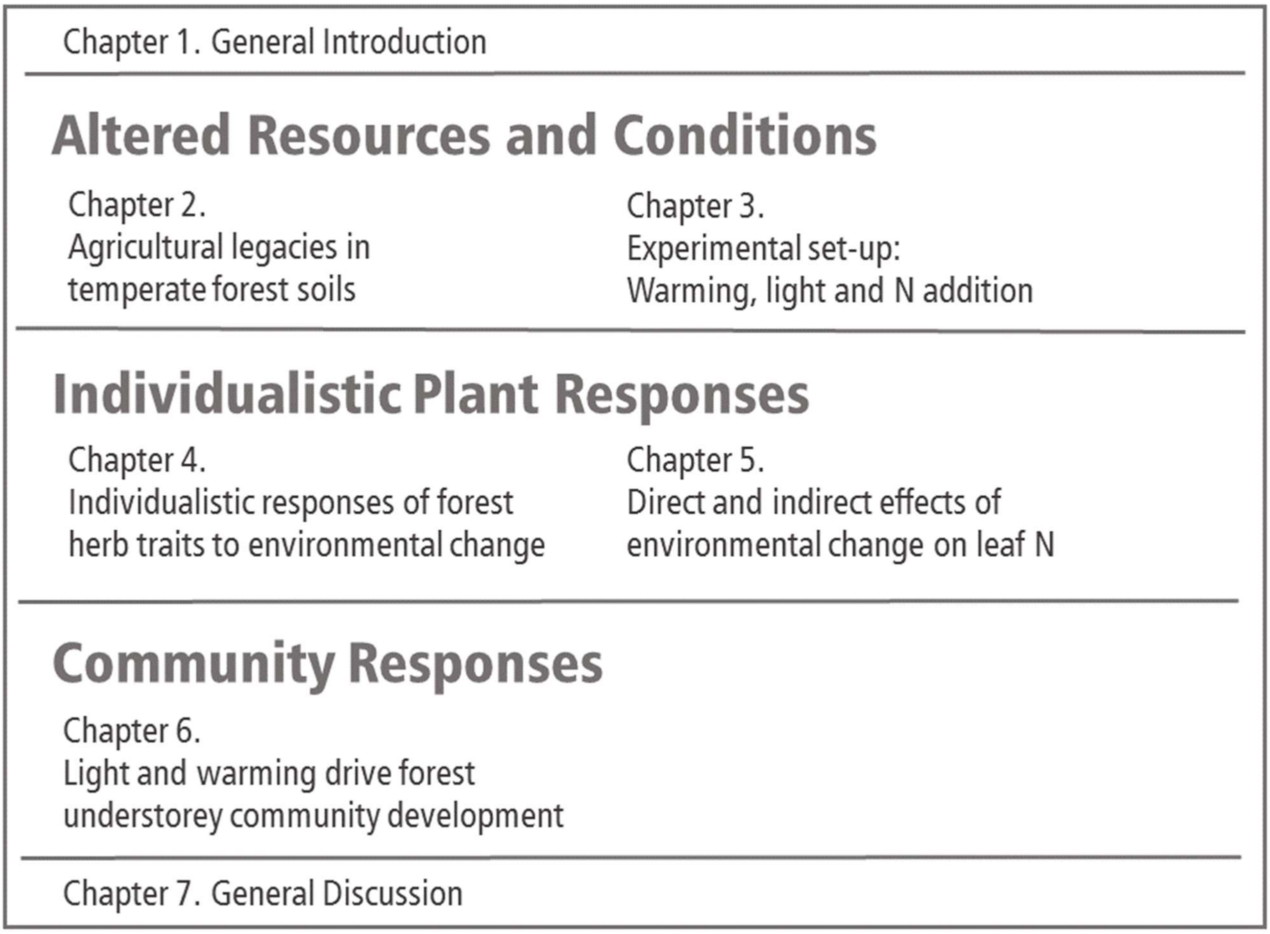
Thesis outline

In this introductory chapter (***Chapter 1***), the main structure and ideas of this thesis were outlined, moving from altered resources and conditions due to global change, to individualistic plant responses, followed by a community response. The following chapters also adopt this structure.

Firstly, we aimed to understand the altered resources and conditions that these common global change drivers can engender in temperate forest ecosystems. ***Chapter 2*** investigates the dependence of agricultural legacies in temperate forest soils on gradients of soil fertility, N deposition and regional P management. ***Chapter 3*** describes the experimental methods that we applied to herbaceous communities planted on these soils, with treatments of warming, illumination and N enrichment.

Secondly, we investigated individualistic plant responses to the multiple treatments. ***Chapter 4*** studies intraspecific trait variation (ITV) of forest herbs in these global change contexts. ***Chapter 5*** describes the direct and indirect effects of the treatments on leaf N, including results from a novel N tracing method. As a final research chapter, ***Chapter 6*** explores whether individualistic plant species' trait responses scale up to a community-level change in traits, by accounting for species reordering and ITV.

Finally, the overarching results of altered resources and conditions, individualistic plant responses, and community responses to these changes are presented in the General Discussion (***Chapter 7***). These overarching findings can provide advances in the field of global change ecology, and support informed forest management and forest policy.



Chapter 1. General Introduction

Altered Resources and Conditions

Chapter 2.
Agricultural legacies in
temperate forest soils

Chapter 3.
Experimental set-up:
Warming, light and N addition

Individualistic Plant Responses

Chapter 4.
Individualistic responses of forest
herb traits to environmental change

Chapter 5.
Direct and indirect effects of
environmental change on leaf N

Community Responses

Chapter 6.
Light and warming drive forest
understorey community development

Chapter 7. General Discussion

Figure 1.7. The chapters in this thesis are structured progressively to reflect the hierarchical response framework of plant community response to environmental change (Smith et al., 2009). This framework was presented here in the General Introduction (**Chapter 1**). The following two research chapters explore altered resources and conditions engendered by agricultural land-use history (**Chapter 2**) and environmental changes of warming, light availability and N addition (**Chapter 3**). Plant responses to these altered resources and conditions are then investigated from an individualistic perspective. **Chapter 4** explores whether forest herb traits respond indivualistically to environmental change. **Chapter 5** explores the importance of forest herbs to N cycling, by investigating direct and indirect effects of environmental change on foliar N. These individualistic plant responses can provide insight into the community-level trait response over time, with taking the processes of species reordering and ITV into account. **Chapter 6** investigates these joint processes on change in community-level traits due to the altered resources and conditions, and shows that light has an important role to play in development trajectories of forest understorey communities. The General Discussion (**Chapter 7**) describes the overarching findings and implications of these results.

Image: Sofie Verstraelen

Chapter 2

Context – dependency of agricultural legacies in temperate forest soils

Adapted from: Haben Blondeel, Michael P. Perring, Laurent Bergès, Jörg Brunet, Guillaume Decocq, Leen Depauw, Martin Diekmann, Dries Landuyt, Jaan Liira, Sybryn L. Maes, Margot Vanhellemont, Monika Wulf, Kris Verheyen (2019). *Context-dependency of agricultural legacies in temperate forest soils*. Ecosystems (22:4); 781 - 79

Abstract

Anthropogenic activities have affected forests for centuries, leading to persistent legacies. Observations of agricultural legacies on forest soil properties can be site-specific and contrasting in numerous observational studies across geographic gradients. Sites and regions vary along gradients in intrinsic soil characteristics, phosphorus (P) management and nitrogen (N) deposition which could affect the magnitude of soil property responses to past cultivation. A single investigation along these gradients could reconcile contradictions and elucidate context-dependency in agricultural legacies. We analysed soil from 24 paired post-agricultural (established after approximately 1950) and ancient forest (in existence before 1850) in eight European regions. Post-agricultural forest soil had higher pH, higher P concentration and lower carbon (C) to N ratio compared to ancient forest. Importantly, gradients of soil characteristics, regional P surplus and N deposition affected the magnitude of these legacies. First, we found that three soil groups, characterising inherent soil fertility, determined extractable base cations, pH and concentrations of total N, organic C and total P. Second, regions with greater current P surplus from agriculture correlated with the highest P legacy in post-agricultural forests. Finally, we found that N deposition lowered pH across forests and increased total N and organic C concentrations in post-agricultural forest. These results suggest that 1) legacies from cultivation consistently determine soil properties in post-agricultural forest and 2) these legacies depend on regional and environmental context, including soil characteristics, regional P surplus and N deposition. Identifying gradients that influence the magnitude of agricultural legacies is key to informing how, where and why forest ecosystems respond to contemporary environmental change.

Introduction

Human activities have profoundly affected ecosystems and biodiversity on the long term (Vellend et al., 2017; Waters et al., 2016). Legacies of past anthropogenic disturbances can obscure ecosystem responses to current disturbance regimes due to time-lags (Bürgi et al., 2017) and potentially interact with other global change drivers to steer ecosystem patterns and processes (Perring et al., 2016). It is possible for numerous accounts of agricultural legacies in forest soil properties to be contrasting, and site specific when compared to each other (Baeten, 2010). Combining observations of agricultural legacies in temperate forest within a single study across regions that vary in gradients of soil characteristics, intensity of agricultural use (phosphorus management) and nitrogen deposition offers the chance to reconcile contrasting findings (Verheyen et al., 2017) and elucidate context-dependency in soil responses to agricultural legacy.

Key soil properties for plant growth show contrasting agricultural legacies

Legacy of agriculture is highly variable and depends on specific management practices that are followed during the agricultural period (Brudvig et al., 2013; McLauchlan, 2006). Agricultural practices can leave imprints in forest soil properties for centuries (Verheyen et al., 1999) and even millennia (Dupouey et al., 2002). We focus on five chemical soil properties that are of high importance for plant growth and where previous research has shown differences in the magnitude, or even in the direction, of their responses to prior agriculture in temperate forest. These are soil organic carbon (C), total nitrogen (N), base cations (calcium (Ca), magnesium (Mg), sodium (Na) and potassium (K)), pH and total and bio-available phosphorus (P).

Comparisons of concentrations of soil organic C and total N between post-agricultural and continuously forested land (ancient forest) are variable. A lower C concentration and C:N has been observed in post-agricultural forest (Foote & Grogan, 2010; Verheyen et al., 1999; Yesilonis, Szlavecz, Pouyat, Whigham, & Xia, 2016), as well as solely a lower C in post-agricultural forest (Falkengren-Grerup, ten Brink, & Brunet, 2006; Leuschner et al., 2014) and even no difference in C and C:N between post-agricultural forest and ancient forest (Compton & Boone, 2000; Koerner, Dupouey, Dambrine, & Benoit, 1997). Comparisons of base cation stocks are even more variable as they can differ within post-agricultural forest sites of single studies (Flinn et al., 2005; Verheyen et al., 1999), likely due to variations in spatial distribution of nutrients (Fraterrigo, Turner, Pearson, & Dixon, 2005) and bulk density (Bizzari et al., 2015). Despite variable base cation concentrations, a consistently higher pH in post-agricultural forest occurs due to past fertilisation and liming in post-agricultural forests (Wall & Hytönen, 2005), but the magnitude of the difference varies (Falkengren-Grerup et al., 2006; Grossmann & Mladenoff, 2008; Koerner et al., 1997; Verheyen et al., 1999; Yesilonis et al., 2016). Aside from raising pH, past fertilisation also leads to a better retention of total phosphorus (P) in forest soils after tillage (Macdonald et al., 2012) in contrast to other disturbance-types such as fires or clearcutting (Bizzari et al., 2015; Grossmann & Mladenoff, 2008) and extensive pasture (Compton & Boone, 2000).

Soil characteristics, phosphorus (P) nutrient management and nitrogen (N) deposition could alter soil responses to agricultural legacy

We identify three gradients that could influence comparisons between soils in post-agricultural and continuously forested sites across multiple regions and thus help explain contrasting site-specific responses.

Firstly, land for cultivation commonly occurs on the richer soils within a given region which may influence soil fertility between ancient vs post-agricultural forests (Flinn et al., 2005). Factors that would determine the suitability for agriculture are soil texture, wetness, slope, aspect, soil depth and underlying bedrock or parent material. Key topsoil processes are additionally affected by biotic components such as identity of tree species and litter quality in forest stands (Cools et al., 2014; De Schrijver, De Frenne, et al., 2012; Nitsch, Kaupenjohann, & Wulf, 2018; Vesterdal, Schmidt, Callesen, Nilsson, & Gundersen, 2008). Both the abiotic suitability of the site for agriculture and soil-forming biotic processes are local-scale drivers that can cause variability among sites within regions unless explicitly controlled for in a study design, preferably with a paired approach. Furthermore, variations in soil characteristics between regions arise along large spatial gradients as nutrient availability generally decreases in temperate regions at higher latitudes and altitudes (De Frenne et al., 2013).

Second, the intensity of past fertilisation and associated nutrient management will determine the magnitude of legacies' persistence in the forest ecosystem. Region-specific nutrient management practices can influence the magnitude of response in soil properties more than the actual agricultural land-use type. Macdonald et al. (2012) illustrated this by showing that soil P legacies arose more prominently across regions than between types of agricultural land use practiced prior to abandonment within each region. The regional phosphorus balance could reflect how nutrient management of phosphorus occurs within sites at a regional level. This is because the region is a collection of local farms, where each farm reports its total amount of P applied on fields, as well as its total outflow from harvest and grazing (see the report to the European Commission by Bomans et al. 2005). Farm practices in regions with a surplus of P have often included excessive manuring of fields and meadows (Ringeval et al., 2017) leading to long-term accumulation of P with major consequences for the environment and global nutrient management (Bouwman et al., 2017; Powers et al., 2019; Rowe et al., 2016; Sattari, Bouwman, Giller, & van Ittersum, 2012). Beyond altering the soil chemisty, past agricultural practices such as ploughing can increase bulk density via compaction (Brudvig et al., 2013; Fraterrigo et al., 2005). Ploughing can also change texture of soil layers by increasing erosion of particular particle sizes (Yesilonis et al., 2016), or via perturbations of the horizontal stratification of soils (Lukac & Godbold, 2011).

Finally, N deposition is a global change driver that varies regionally and originates from intensive agriculture (fertilisation and animal husbandry) and burning of fossil fuels (Bobbink et al., 2010). Acidification and eutrophication are effects of reactive N that influence ecosystem composition and function (De Schrijver et al., 2011) at an ecosystem-specific critical load (Bobbink et al., 2015). Critical loads for different deciduous forest types range from 10 to 20 kg N ha^{-1} y^{-1} (Bobbink et al., 2010, 2015; Simkin et al., 2016). Exceedance of critical loads potentially leads to leaching of compounds following acidification (Bobbink et al., 2010). Eutrophication of the soil occurs by an enrichment of N (Bobbink et al., 2010). This enrichment can lead to nutrient imbalances in plants, e.g. in chronic shortages of P (Tao & Hunter, 2012) unless supply of P is enhanced through other mechanisms such as increased phosphatase activity (Perring, Hedin, Levin, McGroddy, & de Mazancourt, 2008). Nitrogen enrichment is therefore expected to alter P dynamics and legacies of prior fertilization in post-agricultural soils.

Hypothesis: legacies of prior agriculture in temperate forest are context-dependent

Based on the published literature, we expect legacies of prior agriculture for five chemical soil properties important for plant growth when combining measurements from paired sites of post-agricultural and continuously forested land (ancient forest). More precisely, we expect higher concentrations of P and base cations, higher pH, lower concentrations of C and lower C:N in post-agricultural forest. The magnitude of these legacies are expected to be affected by gradients of soil characteristics, agricultural intensity and N deposition thus exhibiting a context-dependency:

1. Inherent soil characteristics relate to texture and underlying parent material of soil deposits, but equally to other edaphic factors such as wetness, relief and exposure. We expect that several variable observations of soil legacies (such as responses of base cations) between pairs of sites and multiple regions are attributable to differences in soil characteristics.
2. The magnitude of the cultivation legacy in post-agricultural forest soil will for one be relative to prior fertilisation intensity and nutrient management on the regional level. Such intensity could be reflected in concentrations of P due to its biogeochemical properties and potential of prolonged adsorption in the soil. In the absence of historical

data, we test whether contemporary regional P surplus is associated with a higher legacy in soil P concentrations in post-agricultural forests compared to the regions' ancient forests.

3. N deposition varies widely on the regional level, where we expect that higher rates of deposition increase responses of acidification (pH) and eutrophication (N), with possible side effects on P availability dependent on the land-use history of the site.

Materials and Methods

Selection of regions along gradients of soil characteristics, regional P balance and N deposition

We selected eight regions across gradients of soil characteristics, regional P balance (surplus of P) and N deposition within temperate Europe (Figure 2.1). These regions span from Pärnu county in the Lääne-Eesti department in Estonia (N 58° 8' 45.1") to the Loiret department (N 47° 50' 10.05”) in France (Appendix Table A2.1.1). We define a region as a large-scaled area with homogeneous macro-climatic conditions (mean annual temperature and precipitation) and topography (Table A2.1.1). For this purpose, we adopt the third level of the *Nomenclature of Territorial Units for Statistics* (NUTS) by the European Union (2015) for our regional boundaries. We aggregated multiple NUTS-III entities to one region where the administrative boundaries were too detailed or where forest patches were on the border of two neighbouring entities (Table A2.1.1).

Invariable soil characteristics such as texture and underlying parent material of soil deposits can differ between post-agricultural and ancient forest within and across regions. To isolate legacy effects of prior agriculture in our comparison of ancient and post-agricultural forest soils, rather than detecting that sites with agricultural history occur on richer soils, we utilised a paired-plot approach within regions (Bizzari et al., 2015; Brudvig et al., 2013; Foote & Grogan, 2010). Thus, we attempted to ensure that local inherent soil characteristics varied minimally within a given pair (Table A2.1.1, World Reference Base (WRB) soil classifications) while simultaneously controlling for overstorey composition (de la Peña et al. 2016, Appendix Table A2.1.2). Pairs and Regions were allowed to differ in inherent soil characteristics. These gradients in soil properties allow to test their importance for key soil chemical variables.

Agricultural intensity on the regional level is estimated by the nutrient balance for P from a report of the Soil Service of Belgium for the year 2003 to the European commission (Bomans et al., 2005). This is calculated per region on NUTS-II and III-levels as the total inflow of P that farmers report to their local governments (fertilisation and manure production) subtracted with the total outflow (harvest and grazing), formulating a regional nutrient balance when expressed per area of agricultural land (kg P ha^{-1}). A positive balance (surplus) indicates an excess of P with potential risk of accumulation and eutrophication by leaching. A negative or zero balance indicates a potential depletion of nutrients. In absence of historical data for P nutrient balances in European regions, we resort to the use of the contemporary values for the P nutrient management in these areas (Bomans et al., 2005). We expect that the relative differences of the P balance between the regions would still hold when using contemporary values for a historical context. This means that we assume that regions with the highest current surplus likely also had the highest P surplus during the mid-20th century. This assumption would, however, be worthy of further investigation. To be careful in the analysis regarding this assumption, we only use P-balance as a predictor to analyse soil P concentrations and not other soil properties.

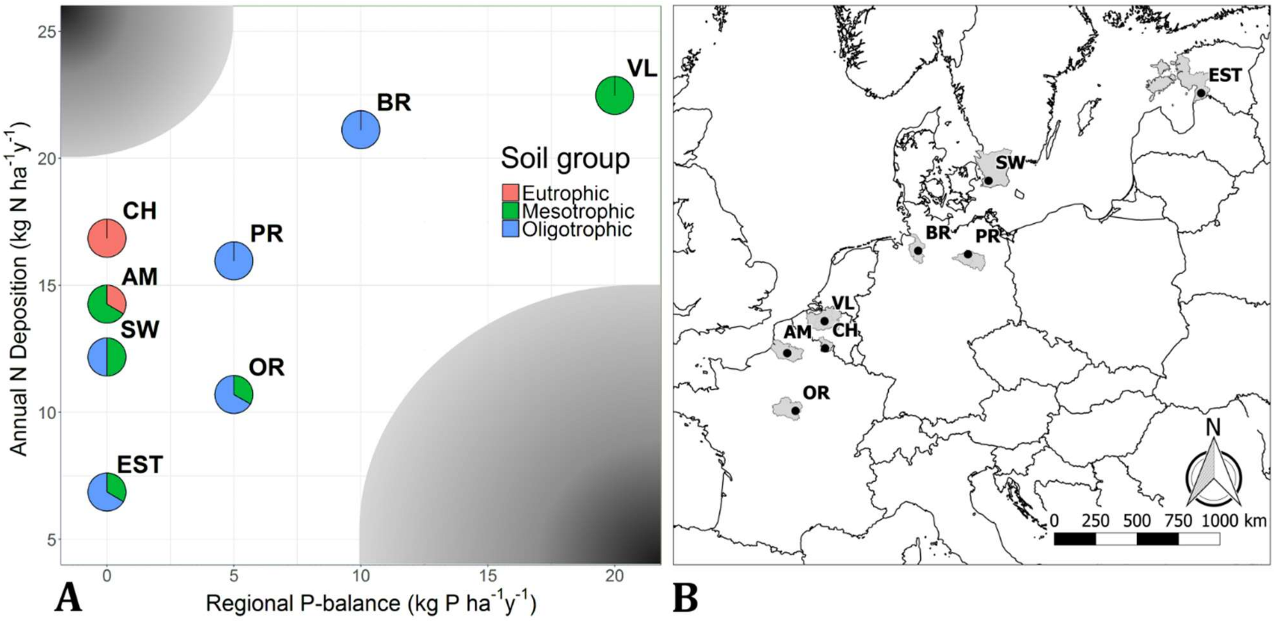


Figure 2.1. Design figure of region selection where three variables provide the context for soil property responses to prior agricultural land use. **Panel A**. Soil group, Regional P balance, N deposition provide the space for environmental context in agricultural legacies. Soil group is a variable on the site level with three classes that group soil properties related to texture and underlying calcareous bedrock. Each of the pie charts represents the proportion of soil groups out of a total of six sites per region. N deposition and Regional P balance are continuous variables that vary on the regional level. Grey zones represent values that are deemed unlikely to occur in forest areas with agricultural history in Western Europe. **Panel B**. Locations of the eight regions with the following region codes: EST= Southern Estonia, SW = Southern Sweden, BR= Bremen (Germany), PR = Prignitz (Germany), VL = Vlaanderen (Belgium), CH= Chimay (Belgium), AM = Amiens (France), OR = Orléans (France). Corresponding NUTS codes for these regions are given in Table A2.1.1.

The magnitude of N deposition between 1990 and 2010 has proven to be an important determinant of the adverse effects of reactive N in forest ecosystems (Dirnböck et al., 2017). Interpolated values of model results from the EMEP database (version 2013, http://www.emep.int/) for the year 2000 were therefore used as the annual N deposition variable. The critical load concept highlights that we have robustly covered ecologically important variation in our choice of sites. For instance, two regions (OR, EST) are found below typical critical load exceedances while, at the other extreme, two regions (VL, BR) have N deposition well in excess of a temperate forest understorey threshold of 18 kg N ha^{-1} y^{-1} (Bobbink et al., 2015; Simkin et al., 2016).

Selection of forest patches and determining their land-use history

We searched for three pairs of ancient and post-agricultural broadleaved forests in each of the eight regions, leading to 48 forest patches included in the study. These pairs consisted of forest patches that are nearby in a landscape context, with the median distance between two pairs being 760 m (Appendix Table A2.1.3). This paired approach allows for minimising differences in site characteristics such as texture, aspect and wetness and allows for isolating the legacies of previous land management rather than inherent differences of post-agricultural and ancient forest sites (Brudvig et al., 2013; Flinn et al., 2005). Forest types were mainly mesophytic with fresh deep soils and sandy to loamy soil textures (see Soil WRB in Table A2.1.1).

The land-use history of these forest patches was determined by use of historical land-use maps (Appendix Table A2.1.4), which pre-dated 1850 for most regions. Forests that have been continuously present on land-use maps since the earliest reliable recording are considered as “ancient” while reforestations on abandoned fields during mid-20th century are considered post-agricultural (G. Peterken, 1996). This “binary” approach (Bürgi et al., 2017) for classifying land-use history types (or land-cover types) has the drawback that subtleties in land management transitions might be missed, potentially leading to contrasting legacies of past agriculture. We minimised this issue by confining the period of cultivation abandonment to around 1950 (Cramer et al., 2008) and by gathering data from multiple regions (Macdonald et al., 2012; Verheyen et al., 2017).

Canopy composition within paired forest patches was ideally as similar as possible and sharing multiple tree species (Appendix Table A2.1.2). The forest canopy often consisted of *Quercus robur/petraea*, *Fraxinus excelsior*, *Acer pseudoplatanus* and *Fagus sylvatica*. Patches with presence of *Alnus* sp. were avoided due to unwanted confounding of N fixation effects, as well as being an indicator for high soil moisture content (idem with *Salix*). Presence of coniferous species was kept at a minimum, but a higher incidence in the northernmost region (EST) was unavoidable.

Soil collection and physicochemical analyses

We collected a large volume of soil (ca 0.1 m^3) in each forest patch from a pit with a depth of 15 cm and surface of 70 x 100 cm. Roots, drainage lines and wet depressions were avoided as a location for sampling in the forest stands. The field campaign ran from October 2015 until February 2016 and its primary purpose was to provide material for a large mesocosm experiment, necessitating the collection strategy used even though composited samples could have been more representative for the entire forest patch. All 48 bulk soil samples were separately sieved (4 mm mesh, 5 mm for heavy soils) for homogenization and removing of coarse organic material. We subsampled 500 ml from the 0.1 m^3 of homogenized soil and processed this through a 1 mm sieve for chemical analysis. Prior to chemical analysis, soil was dried to constant weight at 40°C for 48h.

We analysed samples for pH-H_2O by shaking a 1:5 ratio soil/H_2O mixture for 5 min at 300 rpm and measuring with a pH meter Orion 920A with a pH electrode model Ross sure-flow 8172 BNWP, Thermo Scientific Orion, USA (Norm: ISO 10390:199).

Total C (%) and N (%) concentrations were quantified by combusting samples at 1200°C which releases all C and N and then measuring the combustion gases for thermal conductivity in a CNS elemental analyser (vario Macro Cube, Elementar, Germany). Inorganic C content was measured after 1 g of dry soil was ashed for 4 hours at 450°C by gradually increasing temperature. This procedure drives off organic C leaving only mineral carbon in the ashes, which were measured using a CNS elemental analyser. Subtracting inorganic C from total C gives the organic C (%). This organic C metric was used to calculate the C:N ratio by taking the ratio of organic C to total N.

Extraction of mobile soil cations (Ca, K, Mg, Na, and Al) was performed by extracting soil samples with a 1:5 soil:extractant ratio with ammonium lactacte which consisted of lactic acid (88%), acetic acid (99%) and ammonium acetate (25%) at pH 3.74. The cations were measured using atomic absorption spectrophotometry. The proportion of exchangeable base cations was calculated by converting the values from mg/kg to meq/kg so that charge of the cations is included, and then taking the ratio of the sum $K^{+,}$ Ca^{2+}, Mg^{2+} and Na^+ over the sum of K^+, Ca^{2+}, Mg^{2+}, Na^+ and Al^{3+}.

Total Ca and Fe-concentration was measured by atomic absorption spectrophotometry (AA240FS, Fast Sequential AAS) after complete digestion of the soil samples with $HClO_4$ (65%), HNO_3 (70%) and H_2SO_4 (98%) in teflon bombs for 4 h at 150°C.

All P-concentrations were measured colorimetrically according to the malachite green procedure (Lajtha, Driscoll, Jarrell, & Elliott, 1999). Total P was extracted after complete digestion of the soil samples with $HClO_4$ (65%), HNO_3 (70%) and H_2SO_4 (98%) in teflon bombs for 4 h at 150°C. Soluble and readily soluble P was extracted in $CaCl_2$ (P_{CaCl2}; Simonis & Setatou 1996). Bioavailable P, which is available for plants within one growing season (Gilbert, Gwonig, & Wallace, 2009), was extracted in $NaHCO_3$ (P_{Olsen}; according to ISO 11263:1994(E)).

Soil texture (% Clay, % Sand, % Silt) was analysed with laser diffraction (Coulter Laser LS 13 320 (SIP-050D2) with auto-sampler) after removal of organic material with H_2O_2 (28.5%) and dispersing the sample with Sodium polyphosphate (6%).

Data analysis

All data analyses and handling was performed in R (R Core Team, 2019). Firstly, we clustered data on invariable soil properties related to soil texture and properties of calcareous bedrock (Clay, Silt, Sand and concentrations of total Ca, total Fe and inorganic C) using the *hclust* function in R (R Core Team, 2019). All absolute values of these soil properties can be found in Table A2.2.1. The three resulting clusters from this analysis were used as a categorical variable "Soil group" in the statistical analyses (Appendix Figure A2.2.1). The results from the cluster analysis were subsequently analysed for principal components to check how the soil groups from the cluster analysis align with all centered and scaled continuous variables to aid in interpretation (Figure 2.2).

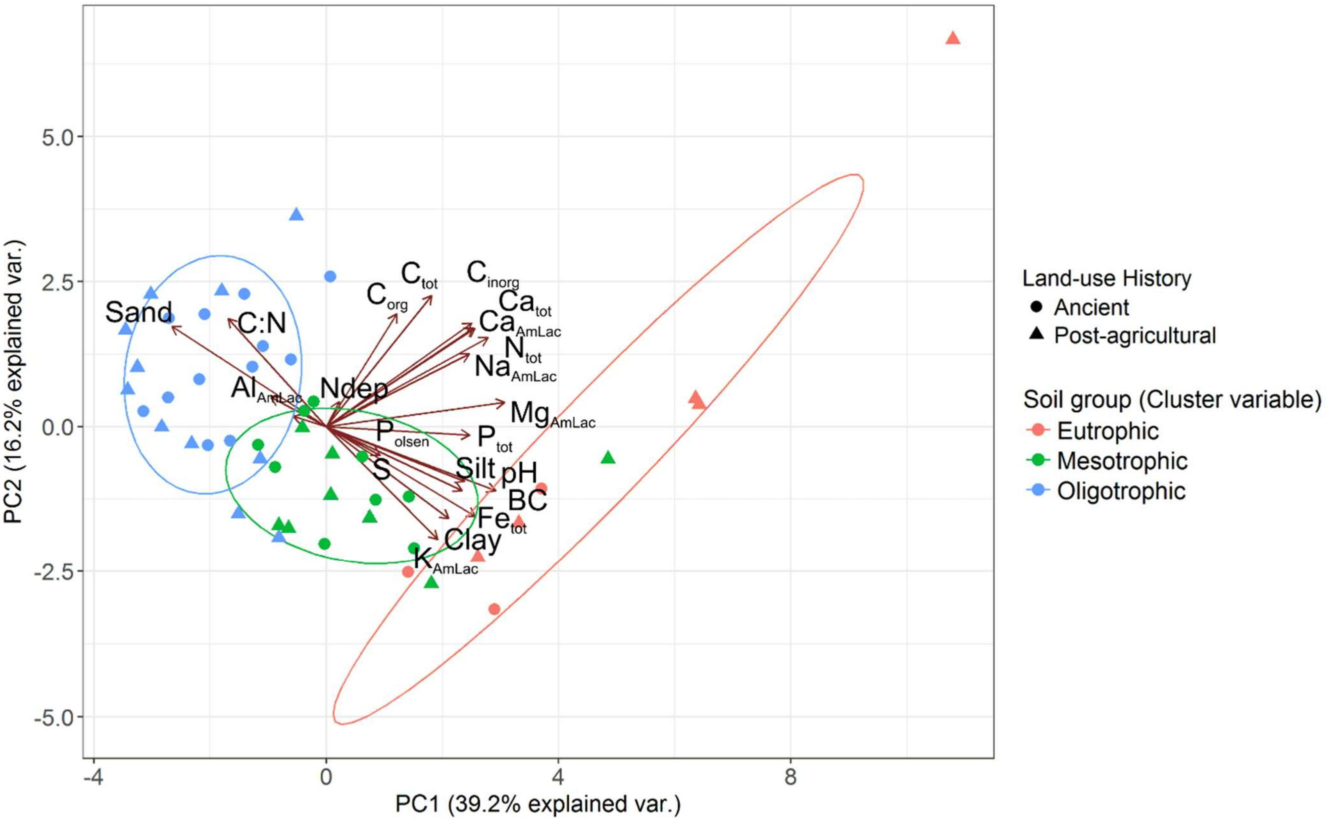


Figure 2.2. Principal component analysis (PCA) showing the three clustered soil groups. Principal component analysis grouped by "Soil Group" (factor variable with three levels resulting from cluster analysis) and marked by land-use history of the soil sample. Oligotrophic soils align most with high sand content and low pH and proportion of extractable base cations (BC). Eutrophic soils are parallel with high Calcium and inorganic C content, which implies high $CaCO_3$ content. Mesotrophic soils adopt an overall intermediate position in the principal component analysis.

In addition, we calculated correlations between all soil variables with Spearman's rank correlation coefficients (Appendix Figure A2.2.2) to aid the interpretation of the soil clustering procedure, the principal component analysis and our a priori selection of response variables. We apply the relative terms 'Eutrophic', 'Mesotrophic' and 'Oligotrophic' to our soil groups as they reflect major differences in soil fertility between our study forests (as in Hirst et al. 2005; Balkovič et al. 2012). These three soil group classes should be considered as a relative classification of inherent soil fertility. It is consequently advised not too compare these classes outside the context of this study. Principal component analysis (Figure 2.2) shows that alignment of Eutrophic soil groups with concentrations of inorganic C and total Ca indicate the calcareous properties of these soil groups, resulting in higher pH and proportion of extractable base cations (BC). Mesotrophic soils adopt an overall intermediate position in soil properties, which is visualised in their position around the origin of the principal component analysis. Oligotrophic soils align with high Sand and a high C:N which both correlate with high acidity and lower nutrient concentration.

We then tested whether land-use history's (LUH) effect on pH, organic carbon (org C), total nitrogen (tot N), C:N, proportion of extractable base cations (BC), total phosphorus (P_{total}) and bio-available phosphorus (P_{Olsen}) was context dependent by considering the gradients of invariable soil characteristics (Soil group), nitrogen deposition (Ndep), and P nutrient management (P-balance). The land use-history of the forest is a categorical variable with two levels indicating whether a forest is continuously forested since at least 1850 (Ancient) or whether a forest has been established around 1950 on abandoned arable land (Post-agricultural). Soil group is a categorical variable with three levels that reflects the inherent soil fertility, as the variable is a product of the cluster analysis on soil properties that relate to texture and calcareous bedrock. To test whether legacies of prior agriculture depend on the soil group, an interaction of LUH*Soil group was added in the explanatory models along with the constituent main effects (hypothesis 1).

Testing the dependence of agricultural legacies in soil properties along the nitrogen deposition gradient was conducted by adding an interaction term of LUH*Ndep (hypothesis 3). These two interaction terms and their main effects formed the fixed factors of the base model (Equation 2.1). The interaction of Ndep and Soil group was not included in this model due to a limited spread of one soil group (eutrophic) along the N deposition gradient.

To test whether total and bio-available phosphorus concentrations in post-agricultural forest are higher in regions with greater P surplus (hypothesis 2), we add in an extra interaction term between LUH*P-balance to model responses of total phosphorus (P_{total}) and bio-available phosphorus (P_{Olsen}). The phosphorus balance could also interact with nitrogen deposition to determine responses of P_{total} and P_{Olsen} (hypothesis 3), so a final term of Ndep*P-balance was added to the model structure of these properties (Equation 2.2). We adopted the use of hierarchical mixed-effects models to test these effects, using the *lme4* package and the function *lmer* (Bates, Mächler, Bolker, & Walker, 2014) with Pair within Region incorporated as a nested random effect. We used maximum likelihood estimation to allow the calculation of a likelihood ratio test when comparing between models.

$$Response\ variable \sim LUH + Soil\ group + Ndep + LUH * Soil\ group + LUH * Ndep \\ + (1|\,Region/Pair)$$

Equation 2.1. Base model

$$P \sim base\ model + P\text{-}balance + LUH * P\text{-balance} + Ndep * P\text{-}balance + (1|\,Region/Pair)$$

Equation 2.2. Expanded base model with P balance as an additional interactive term to test responses of soil P

We then found the most parsimonious models for explaining variation in each response variable using stepwise backwards model selection and a Chi-squared test in the *drop1* function (R Core Team, 2019) for calculation of p-values on the likelihood ratio statistic. We consider $p < 0.05$ as significant and $p<0.1$ as supporting minor evidence for an effect. Non-significant interactions with the highest p-values were left out of the models first, prior to testing the constituent main effects. Main effects were retained, even if non-significant, if they appeared in interaction terms.

Normality of the residuals in the final model was controlled by performing a Shapiro-Wilk test with the *shapiro.test* function (R Core Team, 2019). If normality in the residuals could not be assumed, a log transformation of the response variable was performed for right-tailed response variables and a squared transformation with left-tailed response variables. We then checked the model plots of residuals vs fitted values to check for inconsistencies given the factors included in the model, which we did not observe. We checked for independence of residuals on factor levels via boxplots (for LU, Soil group) and scatterplots for the continuous variables, and did not find any additional skew.

Goodness-of-fit (R^2 values) for linear mixed effects models were calculated using the *r.squaredGLMM* function from the *MuMIn* package (Barton, 2017), which lists both the marginal R^2 (variance explained by fixed factors only) and the conditional R^2 (variance explained by both fixed and random effects, Nakagawa & Schielzeth, 2013).

Results

Post-agricultural forest had significantly ($p<0.05$) higher pH, higher phosphorus concentration (P_{Olsen} and P_{total}) and lower C:N compared to ancient forest (Figure 2.3 and Appendix Table A2.2.2). Soil group affected responses of pH, proportion of extractable base cations and total P concentration ($p <0.05$) as main effects, with the highest values of these three variables in rich "eutrophic" soils and the lowest values in the poor "oligotrophic" soils, with "mesotrophic" soil having intermediate means. Higher N deposition is associated with lower pH ($p <0.05$) across all forest sites as a main effect.

Crucially, we found that gradients of soil characteristics, P nutrient management and N deposition affected the magnitudes of organic C, total N, bio-available Olsen P and total P concentrations in interaction with the forests' land-use history (Figure 2.3, Table A2.2.2).

Firstly, we found minor evidence for a dependence of total N concentration on the land-use history between soil groups ($p <0.1$), as lower total N concentrations in post-agricultural forest only occurred in mesotrophic and oligotrophic soils (Table A2.2.2).

Secondly, we found that the magnitude of phosphorus (P) legacy in post-agricultural forest depended on the regions' phosphorus balance, with higher P concentrations in regions with greater surplus of P (Figure 2.3). The interaction term for land-use history and P balance (which we assume is a proxy for nutrient management intensity of past agriculture) is significant for modelling responses of P_{total} ($p <0.05$) but only with minor evidence for P_{Olsen} ($p <0.1$, see Table A2.2.2).

Finally, we found interactions of N deposition and land-use history on concentrations of organic C ($p <0.05$) but with minor evidence on total N ($p <0.1$, see Table A.2.2). We found higher total N and organic C concentrations in post-agricultural forest with increasing N deposition while total N and organic C concentrations in ancient forest remained unchanged (Figure 2.3).

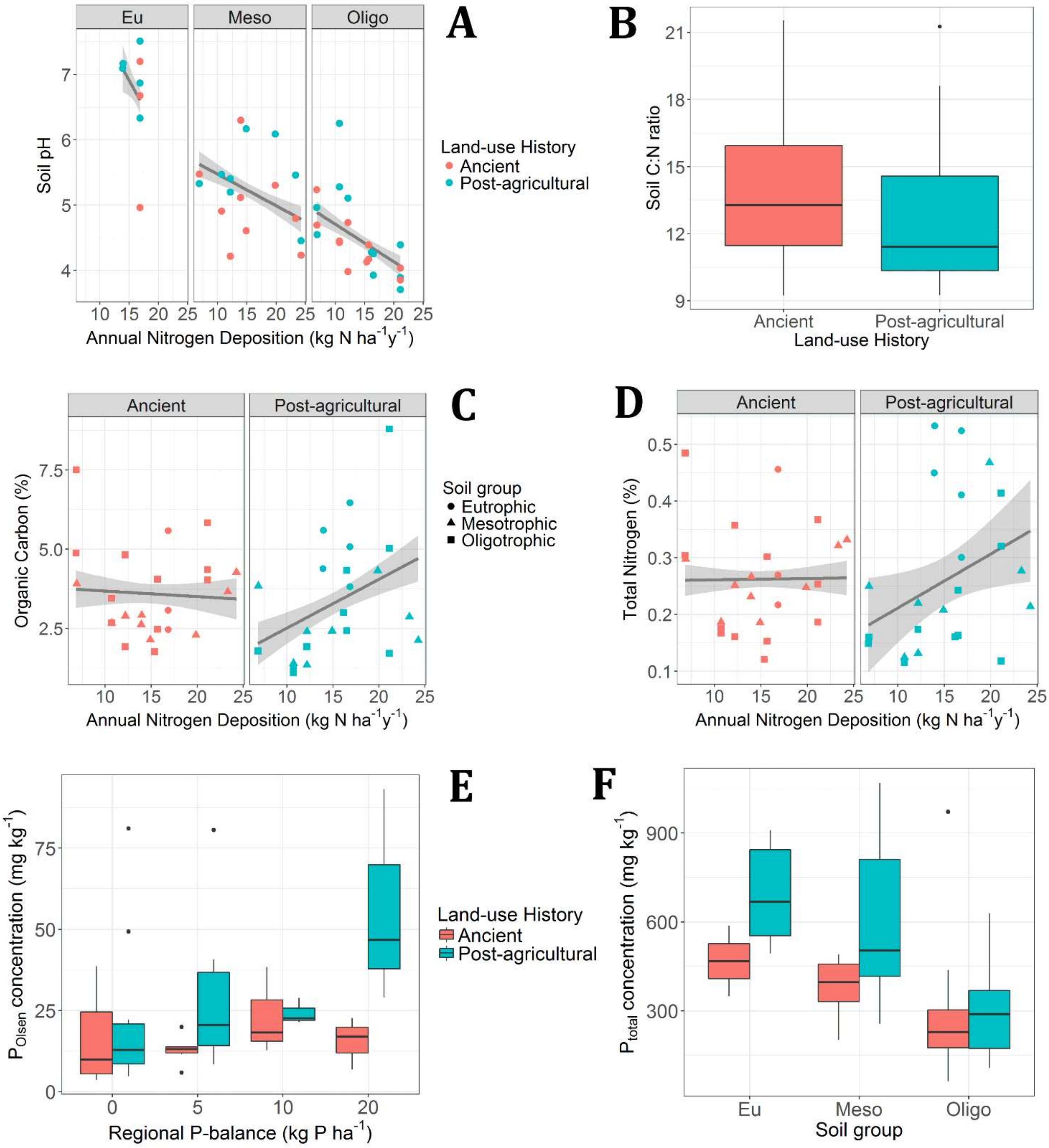


Figure 2.3. Context-dependent legacies commonly shape soil properties in post-agricultural forests. Panel A: Soil pH as a function of nitrogen deposition within each soil group, with post-agricultural forest soils having a higher pH on average ($p<0.05$) and a 95% confidence interval on the computed means. **Panel B**: Boxplot of C:N ratio showing lower values in post-agricultural forest ($p<0.05$). **Panel C:** Organic C (%) increases in post-agricultural forest with increasing N deposition, but no N deposition effect occurs in ancient forest, with a 95% confidence interval on the computed means ($p<0.05$). **Panel D:** Organic N (%) increases in post-agricultural under higher N deposition, but no N deposition effect occurs in ancient forest, with a 95% confidence interval on the computed means ($p<0.1$). **Panel E:** The difference in Olsen P of post-agricultural forest soils compared to ancient forest soil increases with regional P-balance ($p<0.1$). **Panel F:** Difference in concentrations of total P between ancient and post-agricultural forest is dependent on soil group ($p<0.05$). The sorption of P is highest in eutrophic clay and carbonate rich soil, reflecting its biogeochemical nature.

Discussion

Combining soil data from 24 paired sites of ancient and post-agricultural forests across eight European regions successfully elucidated consistent legacies of past land use. As expected, we observed an overall higher P concentration, higher pH, and lower C:N ratio in post-agricultural forest compared to ancient forest. The magnitude of these legacies was affected by gradients of soil characteristics, P nutrient management on the regional level, and N deposition thus exhibiting a context-dependency. First, we found that three soil groups characterised the inherent fertility of the soils and determined the proportion of extractable base cations, pH and concentrations of total N, organic C and total P. Second, regions with greater current surplus of P from agriculture experienced the highest P legacy in post-agricultural forests. Finally, we found that increasing N deposition coincided with a lower pH across forests, and increasing total N and organic C concentrations in post-agricultural forest. These results suggest that 1) land-use legacies from cultivation consistently determine soil property responses in post-agricultural forest and 2) differences in the magnitude of response to land-use history can relate to the regional and environmental context, including soil characteristics, regional surplus of P and nitrogen deposition.

Inherent soil characteristics are important when comparing legacies of prior agriculture between regions and sites as portrayed in the difference in the proportion of extractable base cations, pH and agricultural phosphorus legacies that we observed between the three soil groups (hypothesis 1). Forests on abandoned fields with carbonate and clay-rich soils exhibited the highest total P concentrations (694 mg P/kg), likely due to strong retention of P after cultivation (von Wandruszka, 2006). This result was in contrast to ancient forest on sandy oligotrophic soil (292 mg/kg), as these soils are capable of retaining P only by adsorption with Fe/Al oxides which is generally lower than sorption by clay minerals (Gérard, 2016). The large regional differences in soil characteristics consequently determined the P legacy effect.

The magnitude of phosphorus legacy in post-agricultural forest was furthermore affected by the P balance in the regions (hypothesis 2). Post-agricultural forests had higher total P concentrations (P_{total}, $p<0.05$) and bio-available P concentrations (P_{Olsen} ,$p<0.1$) in regions with greater surplus of P, where fields and meadows are prone to intensive fertilisation (Ringeval et al., 2017). Since P is particularly persistent in soils (Fisher & Binkley, 2000), concentrations of P_{total} are excellent indicators of prior cumulative fertilisation. P_{Olsen} reflects the labile P pool, which is thought to be available for immediate biological uptake (Gilbert et al., 2009), and consists of phosphate in the soil solution or phosphate that can rapidly desorb or mineralise from inorganic or organic soil compounds (De Schrijver, Vesterdal, et al., 2012). In Flanders (Belgium), a region with a 20 kg surplus of P per ha of agricultural land, P_{Olsen} in post agricultural forest was on average 56.3 mg/kg, which was more than triple the 15.5 mg/kg in paired ancient forests. A biological consequence of this dependence of P legacy on regional P balance is that typical forest plants recruit poorly in post-agricultural forest under high nutrient stocks (Baeten, Vanhellemont, De Frenne, Hermy, & Verheyen, 2010; Honnay et al., 2002) and are therefore likely less inhibited in areas with a lower P balance (Brunet, De Frenne, et al., 2012a; Holmes & Matlack, 2017).

Study regions with higher N deposition exhibited responses of acidification and eutrophication in respectively pH and concentration of total N as hypothesized (hypothesis 3). The pH was 0.56 units lower across soil groups for each 10 kg N $ha^{-1}y^{-1}$ of deposition. This acidifying response magnifies the risk that Fe/Al oxides leach in poorly buffered oligotrophic soils (Lukac & Godbold, 2011), underlining that these systems are most susceptible to acidification (Bobbink et al., 2015). Aside from acidification, greater N deposition is associated with increased organic C and total N concentration but only in post-agricultural forest. We identify two possible explanations why we found signals of an accumulation soil organic matter in post-agricultural forest under high N deposition. First, decomposer communities of post-agricultural forests may be less adapted to decomposition in high acidity than decomposer communities of ancient forest (Fichtner et al., 2014; Tardy et al., 2015). Second, it is likely that N deposition has stimulated an acceleration in forest growth and C storage in young temperate deciduous forests (Fowler, Adams, & Peterjohn, 2015; Pretzsch et al., 2014). N deposition thus depends on forest land-use history to affect organic C and total N in forest soils. This dependence could reveal why responses of eutrophication by N deposition were found to be less clear in temperate forests as opposed to other ecosystems such as grassland and heathland (Bobbink et al. 2010; De Schrijver et al. 2011; Verheyen et al. 2012, but see Dirnböck et al. 2014).

Conclusions

Legacies of prior agriculture can be important drivers of global change (Foster et al., 2003) in temperate forest, and can interact with other environmental changes (Perring et al., 2016). Our findings showed that agricultural legacies across temperate forest sites are elucidated when considering differing soil characteristics, regional phosphorus nutrient management and nitrogen deposition on a regional level. Identifying gradients that have influenced the magnitude of agricultural legacies is key to informing how, where and why forest ecosystems respond to contemporary environmental change.

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Appendix 2.1. Additional information on the 48 forests

Table A2.1.1. Additional attributes to the soil samples. The sample code consists of concatenated information on the region, the land-use history and the pair number. AM = Amiens (France), BR= Bremen (Germany), CH= Chimay (Belgium), EST= Southern Estonia, OR = Orléans (France), PR = Prignitz (Germany), SW = Southern Sweden, VL = Vlaanderen (Belgium). The letter "A" and "R" separated by the hyphen respectively symbolizes ancient or recent post-agricultural land-use history and ends with the pair number. NUTS-III codes are administrative regions according to the Nomenclature of Territorial Units for Statistics (NUTS) by the European Union (2015). The "Name" is either the place name of the forest, or the closest village centre. Forest age is based on the historical information in Supplementary information Table A2.1.4. N deposition (kg N ha^{-1}) is data extracted from the EMEP database. The phosphorus balance (P-balance) in kg excess P ha^{-1}. Coordinates are given as longitudes and latitudes, altitude in meters. Mean annual temperature (MAT, °C) and mean annual precipitation (MAP, mm) are extracted from the WorldClim database (http://www.worldclim.org/).The Soil classification is in the taxonomy of the World Reference Base for Soil Resources (WRB) and is given in the last column for information. We classified our soils samples using local site descriptions and local forestry maps or regional soil maps.

Sample	NUTS-III	Land-use history	Name	Age	N dep.	P bal.	Long.	Lat.	Alt.	MAT	MAP	Soil WRB
AM-A1	FR223 - Somme	Ancient	Beaucamps-le-jeune	>230	14.0	0	1.75718	49.82007	181	9.6	710	Eutric Cambisol
AM-A2	FR223 - Somme	Ancient	Gentelles	>230	13.9	0	2.43972	49.84222	120	10.0	658	Eutric Cambisol
AM-A3	FR223 - Somme	Ancient	Mametz	>230	14.9	0	2.7525	50.01992	128	9.8	676	Haplic Luvisol
AM-R1	FR223 - Somme	Post-agricultural	Beaucamps-le-jeune	70	14.0	0	1.7405	49.82923	170	9.7	678	Calcaric Cambisol
AM-R2	FR223 - Somme	Post-agricultural	Gentelles	70	13.9	0	2.42726	49.84303	86	10.1	652	Calcaric Cambisol
AM-R3	FR223 - Somme	Post-agricultural	Mametz	70	14.9	0	2.75352	50.01988	120	9.8	676	Haplic Luvisol
BR-A1	DE937 - Rotenburg	Ancient	Stellingdorf (Ge_W_O_03)	>150	21.1	10	9.40361	53.34333	38	8.4	748	Gleyic Podzol
BR-A2	DE939 - Stade	Ancient	Hohenhausen (Ge_W_O_14)	>190	21.1	10	9.45906	53.3531	38	8.4	749	Gleyic Podzol
BR-A3	DE937 - Rotenburg	Ancient	Weertzen (Ge_W_O_02)	>130	21.1	10	9.396	53.32928	29	8.4	746	Gleyic Podzol
BR-R1	DE937 - Rotenburg	Post-agricultural	Stellingdorf (Ge_W_O_08)	50	21.1	10	9.39556	53.33806	29	8.4	747	Gleyic Podzol
BR-R2	DE937 - Rotenburg	Post-agricultural	Hohenhausen (Ge_W_O_01	20	21.1	10	9.45694	53.34667	36	8.4	749	Gleyic Podzol
BR-R3	DE937 - Rotenburg	Post-agricultural	Weertzen (Ge_W_O_44)	60	21.1	10	9.41773	53.33144	34	8.4	752	Gleyic Podzol
CH-A1	BE326 - Arr. Thuin	Ancient	Bois de Salles	>240	16.9	0	4.25806	50.08051	233	9.4	933	Rendzic Leptosol
CH-A2	BE353 - Arr. Philippeville	Ancient	Les réserves de Dailly	>240	16.9	0	4.40258	50.05262	249	9.2	992	Rendzic Leptosol
CH-A3	BE353 - Arr. Philippeville	Ancient	Gros tienne du Bi	>240	16.9	0	4.44323	50.06725	199	9.6	922	Calcaric Cambisol
CH-R1	BE326 - Arr. Thuin	Post-agricultural/ intensive pasture	Monts trieu de l'air	50	16.9	0	4.25101	50.07163	226	9.3	935	Rendzic Leptosol
CH-R2	BE353 - Arr. Philippeville	Post-agricultural/ intensive pasture	Les réserves de Dailly	50	16.9	0	4.4045	50.05467	218	9.2	992	Rendzic Leptosol
CH-R3	BE353 - Arr. Philippeville	Post-agricultural/ intensive pasture	Gros tienne du bi	50	16.9	0	4.443	50.06801	179	9.6	922	Calcaric Cambisol
EST-A1	EE004 - Lääne-Eesti	Ancient	Tali Reinu	>120	6.9	0	24.68092	58.01923	76	5.4	664	Endogleyic Luvisol
EST-A2	EE004 - Lääne-Eesti	Ancient	Laiksaare	>120	6.9	0	24.81304	58.13339	47	5.4	663	Gleyic Luvisol
EST-A3	EE004 - Lääne-Eesti	Ancient	Kikepera	>120	6.8	0	24.85609	58.26674	37	5.5	665	Endogleyic Luvisol
EST-R1	EE004 - Lääne-Eesti	Post-agricultural	Tali Reinu	60	6.9	0	24.72603	58.034	60	5.3	667	Endogleyic Luvisol
EST-R2	EE004 - Lääne-Eesti	Post-agricultural	Laiksaare	60	6.9	0	24.7729	58.1205	47	5.4	665	Gleyic Luvisol
EST-R3	EE004 - Lääne-Eesti	Post-agricultural	Kikepera	60	6.8	0	24.85907	58.30132	35	5.5	664	Epigleyic Podzol
OR-A1	FR246 - Loiret	Ancient	Nogent North	>170	10.7	5	2.75408	47.83968	152	11.0	679	Eutric Cambisol
OR-A2	FR246 - Loiret	Ancient	Nogent Central	>170	10.7	5	2.76104	47.83252	156	10.8	679	Eutric Cambisol
OR-A3	FR246 - Loiret	Ancient	Nogent South	>170	10.7	5	2.76411	47.83238	148	10.8	679	Eutric Cambsiol
OR-R1	FR246 - Loiret	Post-agricultural	Nogent North	60	10.7	5	2.75408	47.84392	130	11.0	666	Calcaric Cambisol
OR-R2	FR246 - Loiret	Post-agricultural	Nogent Central	60	10.7	5	2.76663	47.83518	129	11.0	672	Calcaric Cambisol
OR-R3	FR246 - Loiret	Post-agricultural	Nogent South	60	10.7	5	2.7664	47.83307	134	10.8	679	Calcaric Cambisol
PR-A1	DE40D - Ostprignitz-Ruppin	Ancient	1.7 km Maulbeerwalde	>230	15.7	5	12.37876	53.17829	111	8.2	584	Haplic Albeluvisol
PR-A2	DE40F - Prignitz	Ancient	2.5 km nne Pritzwalk	>230	15.7	5	12.19625	53.17175	75	8.3	575	Haplic Albeluvisol
PR-A3	DE40F - Prignitz	Ancient	3.1 km sw Meyenburg	>230	15.4	5	12.20848	53.28882	108	8.1	590	Haplic Albeluvisol
PR-R1	DE40F - Prignitz	Post-agricultural	2.5 km sw Putlitz	70	16.5	5	11.96965	53.232	66	8.5	582	Gleyic Albeluvisol
PR-R2	DE40F - Prignitz	Post-agricultural	2.0 km se Putlitz	80	16.2	5	12.05946	53.23826	69	8.4	577	Haplic Albeluvisol
PR-R3	DE40F - Prignitz	Post-agricultural	1 km SW Sagast	70	16.5	5	11.9375	53.25991	79	8.4	581	Haplic Albeluvisol
SW-A1	SE224 - Skåne län	Ancient	Torup (250)	>210	12.2	0	13.20699	55.55432	65	7.7	647	Dystric Cambisol
SW-A2	SE224 - Skåne län	Ancient	Skabersjö (58a)	>170	12.2	0	13.19563	55.54342	50	7.8	640	Dystric Cambisol
SW-A3	SE224 - Skåne län	Ancient	Skabersjö (74b)	>170	12.2	0	13.30391	55.5577	61	7.7	660	Dystric Cambisol
SW-R1	SE224 - Skåne län	Post-agricultural	Torup (251)	80	12.2	0	13.20736	55.5517	71	7.7	647	Dystric Cambisol
SW-R2	SE224 - Skåne län	Post-agricultural	Skabersjö (62a)	60	12.2	0	13.20175	55.54378	57	7.7	648	Dystric Cambisol
SW-R3	SE224 - Skåne län	Post-agricultural	Skabersjö (84l)	70	12.2	0	13.33121	55.54688	63	7.7	669	Dystric Cambisol
VL-A1	BE234 - Arr. Gent	Ancient	Aelmoeseneiebos (5l)	>240	24.3	20	3.80259	50.97501	24	10.2	758	Haplic Albeluvisol
VL-A2	BE242 - Arr. Leuven	Ancient	Doode Bemde (Langerodebos)	>240	19.9	20	4.63983	50.82343	36	10.2	786	Eutric Cambisol
VL-A3	BE211 - Arr. Antwerpen	Ancient	Muizenbos	>240	23.3	20	4.57108	51.19906	20	10.0	781	Gleyic Albeluvisol
VL-R1	BE234 - Arr. Gent	Post-agricultural/ intensive pasture	Aelmoeseneiebos (5m)	40	24.3	20	3.80207	50.97549	21	10.2	758	Haplic Albeluvisol
VL-R2	BE242 - Arr. Leuven	Post-agricultural	Doode Bemde (Langerodevijver)	50	19.9	20	4.64174	50.82799	28	10.2	781	Eutric Cambisol
VL-R3	BE211 - Arr. Antwerpen	Post-agricultural	Muizenbos	50	23.3	20	4.57092	51.19965	17	10.0	781	Gleyic Albeluvisol

Table A2.1.2. Tree and understorey composition of selected forest patches. We recorded the understorey during soil collection (October 2015 – February 2016), so that only spring geophytes in dormant states and evergreen plant were recognised.

Sample	Land-use history	Forest Name	Forest Age	Trees species	Understorey
AM-A1	Ancient	Beaucamps-le-jeune	>230	*Quercus robur* *Fraxinus excelsior* *Acer campestre* *Corylus avellana*	*Rubus sp.* *Lamium galeobdolon* *Dryopteris filix-mas* *Viola sp.*
AM-A2	Ancient	Gentelles	>230	*Quercus robur* *Corylus avellana* *Fagus sylvatica* *Tilia cordata*	*Anemone nemorosa* *Lamium galeobdolon* *Urtica dioica* *Ajuga reptans*
AM-A3	Ancient	Mametz	>230	*Tilia cordata* *Quercus robur* *Acer pseudoplatanus*	*Hyacintoides non-scripta* *Anemone nemorosa* *Dryopteris carthusiana*
AM-R1	Post-agricultural	Beaucamps-le-jeune	70	*Acer pseudoplatanus* *Sorbus aucuparia* *Corylus avellana*	*Millium effusum* *Hedera helix* *Rubus sp.*
AM-R2	Post-agricultural	Gentelles	70	*Quercus robur* *Corylus avellana* *Tilia cordata*	*Hedera helix* *Ligustrum vulgare* *Sambucus nigra*
AM-R3	Post-agricultural	Mametz	70	*Tilia cordata* *Acer pseudoplatanus* *Corylus avellana*	*Rubus sp.* *Geum urbanum* *Sambucus nigra*
BR-A1	Ancient	Stellingdorf	>150	*Quercus robur* *Fagus sylvatica*	*Lamium galeobdolon* *Stellaria holostea* *Oxalis acetosella* *Galium odoratum*
BR-A2	Ancient	Hohenhausen	>190	*Quercus robur*	*Oxalis acetossela* *Hedera helix* *Rubus sp*
BR-A3	Ancient	Weertzen	>130	*Fagus sylvatica* *Fraxinus excelsior* *Corylus avellana*	*Oxalis acetosella* *Anemone nemorosa* *Hedera helix*
BR-R1	Post-agricultural	Stellingdorf	50	*Quercus robur* *Betula pendula* *Corylus avellana*	*Dryopteris dilatata* *Rubus sp.*
BR-R2	Post-agricultural	Hohenhausen	10	*Quercus robur* *Betula pendula*	*Dryopteris carthusiana* *Rubus sp* *Moehringia trinervia* *Lonicera periclymenum*
BR-R3	Post-agricultural	Weertzen	60	*Betula pendula*	*Lonicera periclymenum* *Rubus sp.* *Poa trivialis* *Moehringia trinervia*
CH-A1	Ancient	Bois de Salles	>240	*Quercus petraea* *Corylus avellana* *Carpinus betulus*	*Rubus sp.* *Lonicera periclymenum*
CH-A2	Ancient	Les réserves de Dailly	>240	*Prunus avium* *Corylus avellana* *Carpinus betulus*	*Rubus sp.* *Lamium galeobdolon* *Arum maculatum* *Hedera helix*
CH-A3	Ancient	Gros tienne du Bi	>240	*Quercus petraea* *Fraxinus excelsior* *Carpinus betulus*	*Lonicera periclymenum* *Fragaria vesca* *Deschapmsia cespitosa* *Rubus sp.*
CH-R1	Post-agricultural/ intensive pasture	Monts trieu de l'air	50	*Quercus petraea*	*Rubus sp.* *Lonicera periclymenum*
CH-R2	Post-agricultural/ intensive pasture	Les réserves de Dailly	50	*Fraxinus excelsior*	*Rubus sp.* *Cardamine flexuosa* *Galium aparine* *Hedera helix*
CH-R3	Post-agricultural/ intensive pasture	Gros tienne du bi	50	*Quercus petraea* *Carpinus betulus* *Corylus avellana*	*Hedera helix* *Arum macculatum* *Lonicera periclymenum*
EST-A1	Ancient	Tali Reinu	>120	*Fraxinus excelsior* *Acer plataniodes* *Ulmus glabra* *Tilia cordata* *Picea abies*	*Mercurialis perennis* *Galium odoratum* *Stachys sylvatica* *Lamium galeobdolon* *Anemone nemorosa*
EST-A2	Ancient	Laiksaare	>120	*Populus tremula* *Betula pendula* *Picea abies*	*Stellaria holostea* *Dryopteris carthusiana* *Millium effusum*
EST-A3	Ancient	Kikepera	>120	*Acer platanoides* *Tilia cordata* *Betula pendula* *Corylus avellana* *Picea abies*	*Hepatica nobilis* *Lamium galeobdolon* *Oxalis acetosella* *Stellaria holostea* *Lathyrus vernus*
EST-R1	Post-agricultural	Tali Reinu	60	*Popumus tremula* *Betula pendula* *Ulmus glabra*	*Fragaria vesca* *Luzula pilosa* *Oxalis acetosella*

Sample	Land-use history	Forest Name	Forest Age	Trees species	Understorey
				Picea abies	*Deschampsia cespitosa*
EST-R2	Post-agricultural	Laiksaare	60	*Betula pendula* *Acer platanoides* *Picea abies*	*Stellaria holostea* *Dryopteris carthusiana* *Oxalis acetosella*
EST-R3	Post-agricultural	Kikepera	60	*Betula pendula* *Corylus avellana*	*Rubus sp.* *Chaerophylum temulum* *Oxalis acetosella*
OR-A1	Ancient	Nogent North	>170	*Quercus petraea,* *Carpinus betulus*	*Sparse*
OR-A2	Ancient	Nogent Central	>170	*Quercus petraea* *Carpinus betulus*	*Sparse*
OR-A3	Ancient	Nogent South	>170	*Quercus petraea* *Carpinus betulus*	*Hedera helix*
OR-R1	Post-agricultural	Nogent North	60	*Quercus petraea*	*Rubus sp.* *Gerianium robertianum* *Hedera helix*
OR-R2	Post-agricultural	Nogent Central	60	*Quercus robur*	*Rubus sp* *Hedera helix*
OR-R3	Post-agricultural	Nogent South	60	*Quercus robur* *Carpinus betulus*	*Hedera helix* *Geum urbanum* *Lonicera periclymenum*
PR-A1	Ancient	1.7 km Maulbeerwalde	>230	*Betula pendula* *Quercus robur* *Quercus rubra* *Carpinus betulus* *Fagus sylvatica*	*Rubus sp* *Viola reichenbachiana*
PR-A2	Ancient	2.5 km nne Pritzwalk	>230	*Quercus robus* *Carpinus betulus* *Fagus sylvatica* *Carpinus betulus*	*Anemone nemorosa* *Stellaria holostea* *Lonicera periclymenum* *Rubus sp.*
PR-A3	Ancient	3.1 km sw Meyenburg	>230	*Quercus robur* *Fagus sylvatica*	*Adoxa moschatellina* *Anemone nemorosa* *Circaea lutetiana*
PR-R1	Post-agricultural	2.5 km sw Putlitz	70	*Betula pendula* *Quercus robur* *Picea abies*	*Oxalis acetosella* *Deschampsia cespitosa* *Dryopteris dilatata*
PR-R2	Post-agricultural	2.0 km se Putlitz	80	*Quercus robur* *Fagus sylvatica*	*Sparse*
PR-R3	Post-agricultural	1 km SW Sagast	70	*Fagus sylvatica* *Fraxinus excelsior* *Quercus robur* *Betula pubescens*	*Adoxa moschatellina* *Avenella flexuosa* *Anemone nemorosa* *Brachypodium sylvaticum*
SW-A1	Ancient	Torup	>210	*Quercus robur* *Acer pseudoplatanus*	*Oxalis acetosella* *Stellaria holostea* *Dryopteris filix-mas*
SW-A2	Ancient	Skabersjo	>170	*Quercus robur*	*Pteridium aquilinium* *Oxalis acetosella* *Sambucus nigra*
SW-A3	Ancient	Skabersjo	>170	*Quercus robur* *Fagus sylvatica*	*Oxalis acetosella* *Stellaria holostea* *Melica uniflora*
SW-R1	Post-agricultural	Torup	80	*Quercus robur* *Acer pseudoplatanus*	*Mercurialis perennis* *Aegopodium podagraria*
SW-R2	Post-agricultural	Skabersjo	60	*Quercus robur* *Corylus avellena* *Fagus sylvatica*	*Sparse*
SW-R3	Post-agricultural	Skabersjo	70	*Quercus robur* *Fagus sylvatica*	*Urtica dioica* *Dryopteris filix-mas* *Geum urbanum* *Stellaria holostea* *Mercurialis perennis*
VL-A1	Ancient	Aelmoeseneiebos	>240	*Quercus robur* *Fagus sylvatica* *Acer pseudoplatanus*	*Anemone nemorosa* *Hedera helix* *Stellaria holostea*
VL-A2	Ancient	Doode Bemde	>240	*Quercus robur* *Tilia cordata* *Acer pseuoplatanus* *Fraxinus excelsior* *Populus sp.*	*Hedera helix* *Anemone nemorosa*
VL-A3	Ancient	Muizenbos	>240	*Populus* *Fraxinus excelsior* *Corylus avellana*	*Rubus sp.* *Lamium galebodolon* *Anemone nemorosa* *Carex sylvatica*
VL-R1	Post-agricultural/ intensive pasture	Aelmoeseneiebos	40	*Acer pseudoplatanus* *Betula pendula*	*Ranunculus ficaria* *Rubus sp.*
VL-R2	Post-agricultural	Doode Bemde	50	*Fraxinus excelsior* *Acer platanoides* *Quercus robur*	*Rubus sp.*
VL-R3	Post-agricultural	Muizenbos	50	*Betula pubescens* *Populus sp.* *Corylus avellana*	*Glechoma hederacea* *Rubus sp.* *Juncus effusus*

Table A2.1.3. Distance between two paired forest patches. Distances between pairs are ranked from closest (above) to furthest. Forest patches in the Prignitz region (PR) are far removed because the six forest patches were widely scattered in the landscape. Pairing in the Prignitz region was performed on canopy characteristics instead.

Ancient	Post-agricultural	Distance (m)	Distance(km)
VL-A1	VL-R1	64.33	0.06
VL-A3	VL-R3	66.46	0.07
AM-A3	AM-R3	72.68	0.07
CH-A3	CH-R3	86.15	0.09
OR-A3	OR-R3	188.34	0.19
CH-A2	CH-R2	266.19	0.27
SW-A1	SW-R1	292.54	0.29
SW-A2	SW-R2	388.33	0.39
OR-A1	OR-R1	471.38	0.47
OR-A2	OR-R2	511.32	0.51
VL-A2	VL-R2	525.14	0.53
BR-A2	BR-R2	730.04	0.73
BR-A1	BR-R1	795.59	0.80
AM-A2	AM-R2	900.49	0.90
CH-A1	CH-R1	1108.63	1.11
BR-A3	BR-R3	1467.32	1.47
AM-A1	AM-R1	1576.19	1.58
SW-A3	SW-R3	2103.32	2.10
EST-A2	EST-R2	2755.02	2.76
EST-A1	EST-R1	3118.99	3.12
EST-A3	EST-R3	3861.62	3.86
PR-A2	PR-R2	11763.19	11.76
PR-A3	PR-R3	18360.78	18.36
PR-A1	PR-R1	27983.44	27.98

Table A2.1.4. Historical maps and references

Region	Country	Earliest recording	Reference works
Amiens (AM)	France	Carte de Cassini (1785)	Carte de Cassini (1785) Carte d'État-Major (1830) Aerial photographs (1947)
Bremen (BR)	Germany	Kurhannoversche Landesaufnahme (1765 -1770)	Kelm (1994) Kolb and Diekmann (2004)
Chimay (CH)	Belgium	Carte de Ferraris (1777)	Carte de Ferraris (1770-1778) Carte Topographique Institut Géographique Militaire (1909) Carte Topographique Institut Géographique Militaire (1970)
Southern Estonia (EST)	Estonia	Topographic map of the Russian Empire (1894)	Topographic map of the Russian Empire (1894-1915) Topographic maps of USSR (1946)
Orléans (OR)	France	Carte d'État-Major (1838)	Carte d'État-Major (1838) Aerial photographs (1950-1965)
Prignitz (PR)	Germany	Schmettau map (1767-1787)	Wulf (2004) Wulf & Rujner (2011)
South-Sweden (SW)	Sweden	Forest maps of Torup (1799) Forest maps of Skabersjö (1839)	Brunet (2003) Brunet and others (2012)
Flanders (VL)	Belgium	Carte de Ferraris (1771)	de la Peña and others (2016)

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Appendix 2.2. Supporting data analysis

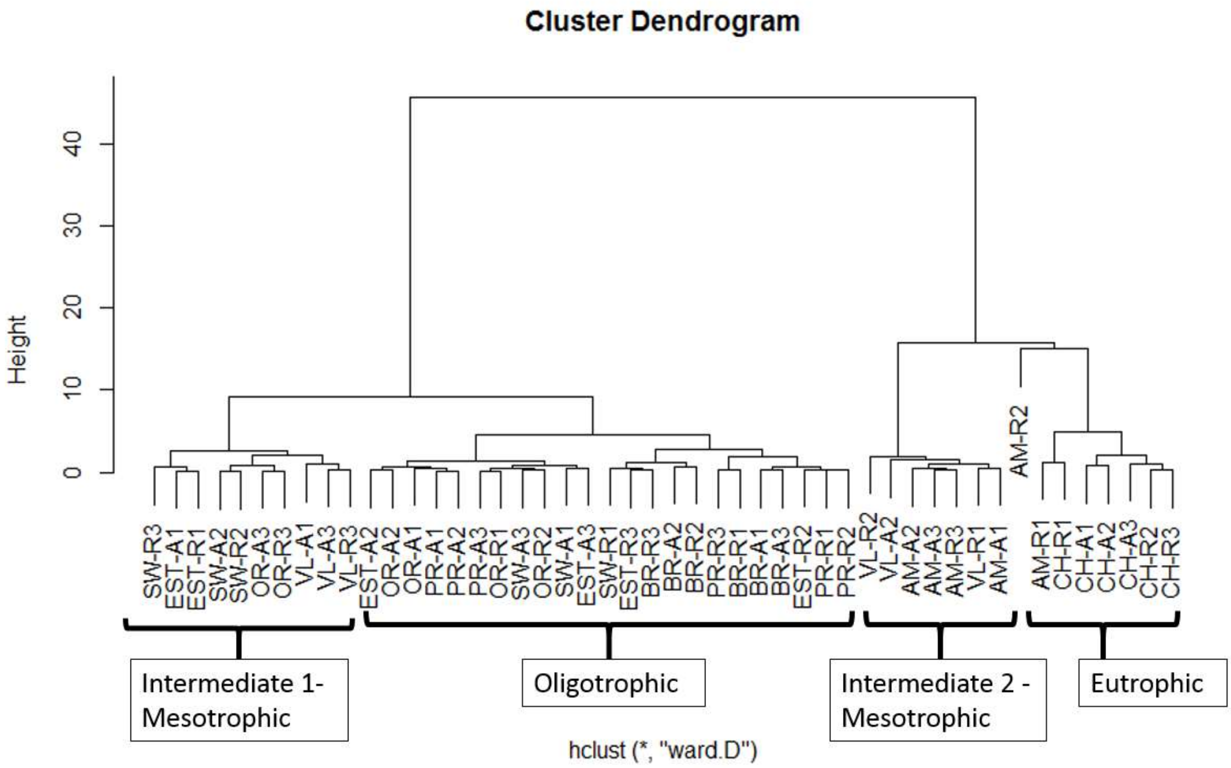


Figure A2.2.1. Cluster dendrogram for soil type classification in three groups. Cluster dendrogram with scaled and centered variables (Fe, total Ca, inorganic C, Silt, Sand and Clay) measured with Euclidean distance using the hclust (*ward.D* method) in R. Two main branches dominate the cluster tree: one branch for richer soils and one for poor soils. Each major branch has an off-shoot that were blocked together for having intermediate properties, hence the grouping in Eutrophic (richest soils), Mesotrophic (intermediate soils) and oligotrophic (poor soils).

	P_{Olsen}	P_{tot}	Ca_{tot}	Fe_{tot}	C:N	K_{AmLac}	Mg_{AmLac}	Ca_{AmLac}	Na_{AmLac}	Al_{AmLac}	C_{tot}	S_{tot}	C_{inorg}	pH-H_2O	Ndep	C_{org}	C:N	Clay	Silt	Sand	BC
P_{Olsen}	1.000	0.120	-0.272	-0.376	-0.209	-0.414	-0.320	-0.199	0.174	-0.094	-0.105	0.225	-0.064	-0.407	0.127	-0.093	-0.033	-0.395	-0.229	0.376	-0.312
P_{tot}		1.000	0.795	0.742	0.462	0.615	0.656	0.517	0.116	0.498	0.236	0.615	0.607	0.669	-0.005	0.201	-0.595	0.468	0.614	-0.614	0.430
Ca_{tot}			1.000	0.829	0.556	0.800	0.913	0.611	-0.160	0.532	0.271	0.485	0.581	0.878	-0.095	0.233	-0.592	0.624	0.666	-0.738	0.742
Fe_{tot}				1.000	0.681	0.836	0.769	0.635	0.041	0.439	0.184	0.439	0.566	0.788	0.038	0.151	-0.557	0.754	0.759	-0.875	0.573
C:N					1.000	0.647	0.608	0.236	-0.107	0.168	0.049	0.174	0.230	0.610	-0.232	0.023	-0.359	0.659	0.549	-0.674	0.551
K_{AmLac}						1.000	0.828	0.567	-0.128	0.528	0.347	0.364	0.488	0.737	-0.124	0.306	-0.429	0.595	0.659	-0.727	0.691
Mg_{AmLac}							1.000	0.592	-0.319	0.518	0.307	0.378	0.549	0.888	-0.040	0.267	-0.536	0.584	0.670	-0.722	0.883
Ca_{AmLac}								1.000	-0.067	0.709	0.553	0.590	0.620	0.441	0.466	0.519	-0.307	0.299	0.579	-0.545	0.479
Na_{AmLac}									1.000	0.088	0.098	0.134	-0.057	-0.229	-0.154	0.137	0.251	0.017	-0.054	0.056	-0.685
Al_{AmLac}										1.000	0.911	0.606	0.727	0.261	0.331	0.898	0.043	0.181	0.470	-0.403	0.326
C_{tot}											1.000	0.366	0.566	0.057	0.290	0.993	0.384	0.012	0.250	-0.174	0.166
S_{tot}												1.000	0.536	0.228	0.342	0.362	-0.331	0.271	0.415	-0.425	0.238
C_{inorg}													1.000	0.449	0.389	0.524	-0.273	0.280	0.600	-0.532	0.448
pH-H_2O														1.000	-0.216	0.019	-0.619	0.667	0.587	-0.704	0.765
Ndep															1.000	0.282	0.047	-0.157	0.241	-0.120	0.074
C_{org}																1.000	0.435	0.006	0.203	-0.137	0.120
C:N																	1.000	-0.355	-0.458	0.473	-0.506
Clay																		1.000	0.511	-0.821	0.474
Silt																			1.000	-0.886	0.542
Sand																				1.000	-0.597
BC																					1.000

Figure A2.2.2. Correlation matrix off all variables with a Spearman's rank correlation coefficient. Correlation coefficients are listed above the diagonal. Below the diagonal is a schematic barplot that is red for negatively correlated variables and blue for positively correlated values. The length of the bar is relative to the maximum value of a correlation coefficient (1).

Table A2.2.1.Values of soil properties used for the statistical analysis. All procedures for measuring these variables can be found in the Methods sections of this chapter. The sample code consists of the region code (see Table A2.1.1), the land-use history ("A" for ancient, "R" for recent post-agricultural) and the pair number. Soil group is a categorical variable with three levels which order "Eutrophic"-" Mesotrophic"-"Oligotrophic" from rich to poor. Clay, Silt and Sand note the relative percentage of each soil texture class (adds up to 100%). pH gives the soil acidity measured as pH H_2O. Tot Ca and Tot Fe were measured after digestion of the samples with a mixture of strong acids (mg $^{-1}$ kg^{-1}). Bio-available concentrations of base cations (Ca, K, Mg, Na) and Al were obtained by digestion in Ammonium Lactate (AmLac, mg $^{-1}$ kg^{-1}), and provide a proportion of extractable base cations (BC, unitless). Soil P concentration was measured as bio-available P from digestion in a weak acid (Olsen P, mg $^{-1}$ kg^{-1}) while total P concentration was measured from a mixture of strong acids (Tot P, mg $^{-1}$ kg^{-1}). Total N (%) and Total C (sum of org C and Anorg C, %) are obtained from a CNS elemental analyser. Available N in NH_4^+ and NO_3^- forms (ISO/TS 14256-1) was measured from a 1 KCl solution. Ammonium (NH_4^+) was determined colorimetrically by the salicylate-nitroprusside method on an auto-analyzer (AA3, Bran Luebbe, Germany). Nitrate (NO_3^-) was determined colorimetrically using the same auto-analyzer after reduction of NO_3^- to nitrite (NO_2^-) in a Cu-Cd column, followed by the reaction of NO_2^- with N-1-napthylethylenediamine to produce a chromophore. This mineral N data was not included in the manuscript because these values may be too sensitive to the moment of sampling (autumn 2015) and may be disturbed by the sieving and mixing during processing. They are added here to be complete and to provide an estimate for N availability, other than C:N which is more linked to soil organic matter.

Sample code	Soil group	Clay	Silt	Sand	pH	Tot Ca	Tot Fe	AmLac Ca	AmLac K	AmLac Mg	AmLac Na	AmLac Al	BC	Tot P	Olsen P	Tot N	Org C	Inorg C	Org C:N	NH^+_4	NO^-_3
AM-A1	Meso	20.1	62.6	17.4	6.3	7684.9	16468.2	4211.4	66.2	85.4	23.9	209.4	1.0	342.6	5.5	0.27	2.92	0.07	10.9	11.0	34.2
AM-A2	Meso	22.3	64.6	13.1	5.1	2319.0	18701.2	1488.6	162.1	144.9	13.7	297.0	0.9	491.3	11.8	0.23	2.62	0.02	11.3	8.2	32.4
AM-A3	Meso	22.8	68.4	8.9	4.6	1034.1	15821.4	352.8	152.7	31.6	5.0	475.2	0.6	396.1	24.7	0.19	2.14	0.03	11.5	3.6	30.2
AM-R1	Eu	33.4	52.8	13.9	7.2	30408.9	39669.0	26327.6	147.0	277.2	51.2	133.2	1.0	843.5	10.3	0.53	5.60	0.74	10.5	23.2	34.4
AM-R2	Eu	11.4	58.6	30	7.1	132147.5	17630.4	105740.0	111.4	465.0	103.3	51.6	1.0	909.2	8.9	0.45	4.39	3.60	9.7	13.4	20.1
AM-R3	Meso	24.7	68.6	6.8	6.2	3132.6	19888.7	2228.6	181.5	118.1	7.6	167.4	0.9	461.2	16.8	0.21	2.42	0.04	11.6	7.1	21.3
BR-A1	Oligo	7.1	21.4	71.5	3.9	829.4	4931.9	395.0	70.3	42.2	9.4	252.6	0.7	262.8	38.4	0.37	5.84	0.04	15.9	5.5	30.9
BR-A2	Oligo	6.9	26.6	66.5	3.9	220.8	850.5	119.0	37.5	23.1	6.5	75.6	0.8	62.0	12.8	0.19	4.03	0.04	21.5	7.6	9.1
BR-A3	Oligo	6	19.1	74.9	4.0	466.7	7128.1	275.8	41.2	35.3	16.7	226.2	0.7	169.4	18.2	0.25	4.35	0.02	17.1	11.4	26.8
BR-R1	Oligo	3.5	10.1	86.4	3.9	167.9	1580.5	91.6	28.5	15.7	3.3	49.2	0.8	106.6	21.4	0.12	1.71	0.04	14.5	5.2	11.0
BR-R2	Oligo	9.3	35.2	55.5	3.7	407.6	1623.1	237.4	74.0	50.7	15.2	142.2	0.8	193.1	28.9	0.41	8.80	0.05	21.3	6.1	18.5
BR-R3	Oligo	4.2	27.6	68.3	4.4	622.3	8913.5	170.0	57.1	57.1	11.4	880.2	0.3	475.8	22.6	0.32	5.03	0.03	15.7	4.1	17.2
CH-A1	Eu	46.4	48.5	5	5.0	678.5	46539.7	467.2	131.0	177.1	12.6	627.6	0.6	349.4	4.1	0.22	2.46	0.06	11.3	14.1	3.1
CH-A2	Eu	40.6	46.5	13	7.2	7961.1	41119.2	6246.0	126.2	126.8	19.3	354.0	1.0	586.7	5.5	0.46	5.58	0.10	12.2	26.8	12.9
CH-A3	Eu	59.7	35.3	4.9	6.7	4116.2	49668.8	3450.0	139.9	117.4	15.6	264.6	0.9	467.1	3.7	0.27	3.07	0.06	11.4	25.8	1.9
CH-R1	Eu	45.7	44.2	10	7.5	30942.5	41231.7	28064.4	184.7	295.7	23.3	184.2	1.0	668.1	13.0	0.52	6.47	1.13	12.3	40.9	2.2
CH-R2	Eu	55.6	37.4	7	6.9	6346.3	37504.0	4935.6	102.5	137.4	13.0	125.4	1.0	493.4	6.4	0.41	5.08	0.07	12.4	28.0	6.9
CH-R3	Eu	55.3	38.1	6.7	6.3	4661.7	40885.9	3814.8	109.0	97.7	13.9	217.2	1.0	553.7	4.8	0.30	3.82	0.08	12.7	18.0	4.8
EST-A1	Meso	27.6	33.3	39.1	5.5	2644.6	11550.8	1609.6	84.3	179.5	5.2	228.0	0.9	484.0	10.8	0.30	3.91	0.07	13.1	11.4	32.0
EST-A2	Oligo	13.3	29.2	57.5	4.7	2569.4	6548.8	1155.4	59.8	68.1	4.8	820.8	0.6	972.0	38.7	0.49	7.51	0.04	15.5	6.1	49.5
EST-A3	Oligo	16.3	24.1	59.5	5.2	2007.3	15504.5	994.8	62.9	164.9	9.7	595.8	0.7	291.6	8.0	0.30	4.88	0.04	16.1	11.5	15.7
EST-R1	Meso	28	35	37	5.3	1694.2	11304.3	980.6	61.4	126.7	9.6	468.6	0.7	286.6	7.8	0.25	3.84	0.03	15.3	13.1	5.4
EST-R2	Oligo	10.3	16.8	73	4.5	478.1	2918.6	170.8	28.1	24.9	3.3	231.0	0.6	226.4	12.8	0.16	1.78	0.02	11.1	16.1	7.8
EST-R3	Oligo	5.9	25.6	68.5	5.0	479.7	6433.5	182.4	70.9	26.9	3.0	350.4	0.5	382.8	20.5	0.15	1.79	0.04	12.0	6.1	17.9
OR-A1	Oligo	18.1	25.2	56.8	4.5	735.2	3198.4	472.6	92.0	57.4	4.8	120.6	0.9	193.7	13.2	0.17	2.68	0.01	16.0	11.8	8.5
OR-A2	Oligo	14.9	30.8	54.3	4.4	429.3	6322.8	222.4	76.5	49.7	6.3	409.8	0.5	177.0	13.1	0.18	3.45	0.02	19.7	17.0	0.3
OR-A3	Meso	16.6	30.2	53.2	4.9	951.9	10955.7	716.8	138.5	104.8	7.1	280.2	0.8	202.1	5.9	0.19	2.67	0.02	14.3	18.3	1.0
OR-R1	Oligo	16.7	20.1	63.2	5.3	885.6	6183.4	713.2	87.8	60.1	4.3	138.6	0.9	336.0	40.8	0.12	1.10	0.01	9.6	8.1	11.7
OR-R2	Oligo	16.4	21.9	61.7	6.3	1878.8	8694.4	1912.0	129.9	60.9	4.9	100.2	1.0	288.7	24.8	0.12	1.20	0.02	10.4	7.0	8.4
OR-R3	Meso	17.9	27.7	54.3	5.5	1627.9	11544.3	1326.6	126.6	75.1	8.1	164.4	0.9	256.6	8.4	0.13	1.40	0.02	11.2	13.8	5.0
PR-A1	Oligo	13.3	26.1	60.5	4.2	504.2	5418.9	125.6	54.5	20.7	5.4	278.4	0.5	149.5	14.0	0.15	2.47	0.02	16.2	3.7	21.0
PR-A2	Oligo	14	24.8	61.2	4.4	1446.2	7403.2	633.0	51.5	62.4	13.0	405.6	0.7	299.0	20.0	0.30	4.06	0.03	13.4	11.3	30.6
PR-A3	Oligo	14.8	21.4	63.9	4.1	476.7	5280.4	90.4	35.7	15.2	4.5	292.2	0.4	179.0	11.6	0.12	1.76	0.02	14.6	12.8	20.3
PR-R1	Oligo	10	20.5	69.5	4.3	323.6	2160.5	169.0	26.9	13.1	9.3	615.6	0.3	151.7	16.4	0.24	4.33	0.03	17.8	12.5	7.9
PR-R2	Oligo	11.3	21.4	67.3	4.3	369.7	4971.1	48.0	42.9	13.1	7.3	683.4	0.2	353.6	80.6	0.16	3.00	0.03	18.6	10.3	0.3
PR-R3	Oligo	5	12.9	82.1	3.9	243.5	1453.0	124.2	23.1	12.6	5.9	106.8	0.7	132.3	13.5	0.16	2.43	0.02	14.9	6.7	7.2
SW-A1	Oligo	12.7	21.3	65.9	4.0	1224.4	11244.4	230.4	71.8	78.2	10.5	528.0	0.5	437.6	24.6	0.36	4.82	0.04	13.5	6.4	45.7
SW-A2	Meso	23.8	26.8	49.5	4.2	1123.7	11149.5	113.6	74.2	29.4	8.4	463.8	0.4	457.7	27.9	0.25	2.90	0.05	11.5	7.2	35.5
SW-A3	Oligo	18.5	23	58.5	4.7	1396.6	8482.6	103.0	51.9	32.9	9.5	345.6	0.5	315.1	9.0	0.16	1.92	0.02	11.9	16.4	8.8
SW-R1	Oligo	4.7	31.8	63.5	5.1	1924.9	11157.8	501.2	91.1	61.4	9.6	378.6	0.7	629.5	49.3	0.17	1.92	0.04	11.1	6.7	15.7
SW-R2	Meso	23.2	26.4	50.3	5.2	1633.9	11596.7	574.2	109.6	83.3	9.2	249.6	0.8	459.2	22.2	0.13	1.34	0.03	10.2	8.2	24.4
SW-R3	Meso	31.9	30.1	38	5.4	3598.6	16159.3	1255.2	123.9	84.3	14.7	346.8	0.8	985.8	81.1	0.22	2.40	0.03	10.9	8.8	25.1
VL-A1	Meso	15.1	46	38.5	4.2	1014.6	10628.2	460.4	72.0	69.5	10.1	326.4	0.7	276.0	22.7	0.33	4.28	0.10	12.9	16.6	21.5
VL-A2	Meso	10.2	62.1	27.8	5.3	2652.9	14829.9	1473.4	47.7	159.4	13.9	135.0	0.9	443.0	6.8	0.25	2.29	0.03	9.2	5.7	38.6
VL-A3	Meso	12.2	35.8	52.1	4.8	1548.9	8873.6	796.0	67.8	52.1	12.6	298.8	0.8	330.6	17.0	0.32	3.66	0.08	11.4	5.2	27.5
VL-R1	Meso	23.1	56.5	20.4	4.5	1339.1	16998.9	621.2	51.8	69.0	12.6	379.2	0.7	752.4	93.2	0.21	2.12	0.06	9.9	7.2	21.8
VL-R2	Meso	15.9	73.6	10.5	6.1	7205.5	30343.6	5165.6	72.5	429.1	27.3	130.2	1.0	1068.9	46.7	0.47	4.33	0.33	9.2	7.7	79.9
VL-R3	Meso	16.2	36.6	47.2	5.5	2507.2	9322.6	1487.0	34.1	15.9	16.1	250.8	0.9	544.2	29.0	0.28	2.86	0.10	10.3	5.3	8.4

Table A2.2.2.Regression coefficients in unit of response variable. Response variables include pH, organic C (org C, %), total N (%), C:N , proportion of extractable base cations (BC), Olsen P-concentration (P_{Olsen}, mg kg^{-1}) and total P-concentration (P_{total} , mg kg^{-1}). Explanatory variables are land-use history (LUH, Ancient or Post-agricultural), Soil group (Eutrophic, Mesotrophic, Oligotrophic), N deposition (Ndep, kg N ha^{-1} y^{-1}) and P-balance (P-bal, kg P ha^{-1}). Regression coefficients and terms of likelihood ratio tests (*LRT*) for main effects and interactions of the most parsimonious model are listed with significant terms ($p<0.05$) in bold. P-values are represented as significance levels (0.1°, 0.05*, 0.01**, 0.001***). Regression coefficients for categorical variables are relative to the intercept, which is 'Ancient' for LUH and 'Eutrophic' for Soil group. Continuous variables (Ndep, P-balance) have slope coefficients. The mean intercepts of the Pair within Region random terms are listed here. Goodness-of-fit is shown as marginal R^2 (only on fixed effects) and a categorical R^2 (includes fixed and random effect terms).

Term	Int.	LUH	Soil group	Ndep	LUH* Soil group	LU* Ndep	P-bal	LUH *P-bal	Ndep* P-bal	R^2_m	R^2_c
pH	7.35	Post-ag +0.405 *LRT* 6.65	Meso -1.49 Oligo -2.31 *LRT* **25.48***	-0.054 *LRT* **8.61**	-	-				0.74	0.74
log(C:N)	5.27	Post-ag -0.10 *LRT* **5.54***	-	-	-	-				0.05	0.59
Org C	5.27	Post- ag -3.08 *LRT* 0.45	Meso -1.88 Oligo -1.21 *LRT* **6.87***	-0.025 *LRT* 1.17		Post-ag *Ndep 0.176 4.55*				0.26	0.33
Tot N	0.34	Post-ag -0.013 *LRT* 0.005	Meso -0.077 Oligo -0.075 *LRT* **13.32***	-0.001 *LRT* 0.805	Post-ag *Meso -0.14 Post-ag *Oligo -0.17 *LRT* 5.53°	Post-ag 0.009 *LRT* 3.06°				0.45	0.50
BC^2	0.89	-	Meso -0.229 Oligo -0.456 *LRT* 14.19***	-	-	-				0.40	0.49
log(P_{Olsen})	2.42	Post-ag +0.293 *LRT* 8.06**	-	-	-	-	0.019 *LRT* 2.59	Post-ag 0.039 *LRT* 2.89°	-	0.25	0.55
log(P_{Total})	6.43	Post-ag +0.047 *LRT* 5.25*	Meso -0.599 Oligo -0.801 *LRT* **6.94***	-	-	-	-0.020 *LRT* 0.264	Post-ag 0.037 *LRT* **4.16***	-	0.26	0.61

Image: Stephanie Schelfhout

Chapter 3

Experimental set-up:

Applying treatments of N addition, warming and enhanced light availability to mesocosms with distinct past land use

Abstract

This methodological chapter summarises the overarching experimental setup, and thus underpins the specific method sections for research chapters 4 – 6. We performed a multi-factor global change experiment to disentangle the effects of multiple environmental change drivers and forest land-use history on herbaceous understorey communities. Our study site is the Aelmoeseneie forest (Belgium), a temperate broadleaved forest under the ownership of Ghent University. We planted 384 mesocosms, consisting of communities with five herbaceous species (four individuals per species) assembled from a pool of 15 forest herbs with varying colonisation capacities and affinity to forest habitat. We overcame dispersal limitation by planting these mixtures of slow and fast colonising species on soils from both ancient forest (forested since at least 1850) and post-agricultural forest (forested since 1950 on former arable land). These soils were collected from eight European regions across gradients of N deposition, agricultural intensity on the regional level, and soil types (Chapter 2). The mesocosms were exposed to two-level full-factorial treatments of warming, light addition (representing changing forest management) and nitrogen (N) enrichment. This design allows to disentangle multiple environmental change effects on herbaceous understorey development. The experiment started in April 2016 with data collection ending in September 2018, so that three consecutive growing seasons were monitored. Survival counts across species showed that 45% of individuals survived until the second growing season (2017), with an average persistence of about two or three species in a mesocosm. The number of survivors did not depend on any treatment effect (including two-way interactions) in any species. There were, however, large differences in mortality between species. The purpose of this methodological chapter is to support further plant trait analyses, moving from within species responses to environmental change (Chapters 4 and 5) and to the community response (Chapter 6).

Site description

The study was conducted in the long-term ecological research site (LTER) Aelmoeseneie forest (50°58′30” N, 3°48′16” E, 20 m a.s.l), an ancient temperate mixed deciduous forest in ownership of Ghent University in Gontrode, Belgium (DEIMS-SDR Database 2019). The region has an Atlantic maritime climate with a mean annual temperature (MAT) of 10.6 °C and mean annual precipitation (MAP) of 768 mm (DEIMS-SDR Database 2019, ICP forest). The tree canopy (90% cover) is dominated by *Fagus sylvatica, Quercus robur, Acer pseudoplatanus, Fraxinus excelsior* and *Larix decidua.*

The in situ manipulations on the mesocosms occur in a 1.8 ha fenced area within the forest. This area in the Aelmoeseneie forest hosts a 35 m tall measuring tower and an intensive monitoring plot for air pollution monitoring (ICP forest programme level II). Data from this air pollution monitoring plot shows a declining N deposition since 1995, which stabilised at 15.5 kg N ha^{-1} in 2014 (Figure 3.1). While these deposition values have declined steadily over the years, the N deposition in 2014 still falls within the critical load (10- 20 kg N ha^{-1} y $^{-1}$) for temperate broadleaved forest (Bobbink & Hettelingh, 2010; van Dobben, Bobbink, Bal, & Hinsberg, 2000). This critical N load indicates that N deposition in our site can induce adverse effects of eutrophication and acidification (Bobbink et al., 2015), which can eventually drive species loss in herbaceous communities (De Schrijver et al., 2011; Dirnböck et al., 2014; Simkin et al., 2016).

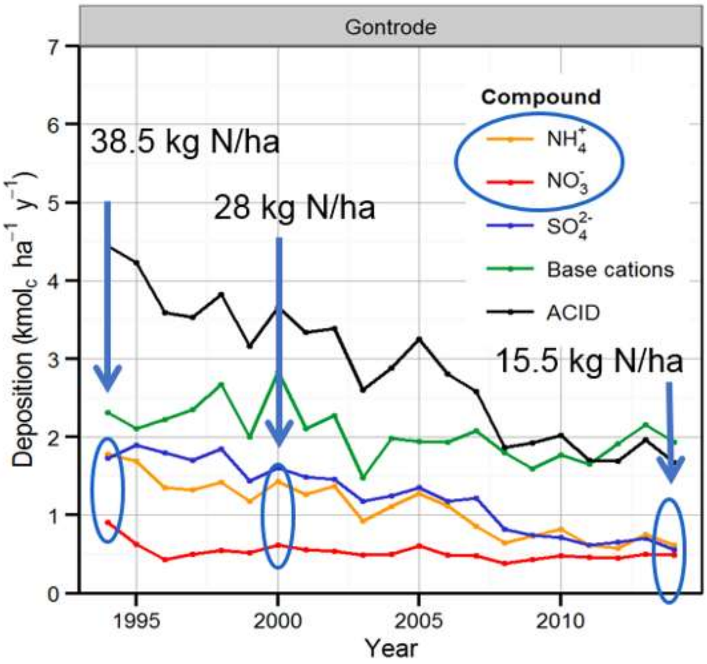


Figure 3.1. Declining acidifying depositions in the Aelmoeseneie forest. The level II plot on air pollution monitoring runs in the Aelmoeseneie forest (Gontrode) since over 20 years. Data from ICP (www.icp-forests.net) and figure from Arne Verstraeten (INBO). The arrows and ellipses highlight the deposition of NO_3^- and NH_4^+ recalculated as kg N ha^{-1}in 1994 (38.5 kg N ha^{-1}), 2000 (28 kg N ha^{-1}) and in 2014 (15.5 kg N ha^{-1}).

Land-use legacies: ancient and post-agricultural forest soils across environmental gradients

In this mesocosm experiment, we used soil from eight temperate European regions collected across gradients of inherent soil fertility, regional phosphorus balance and nitrogen deposition (further details in Blondeel et al. 2019 and Chapter 2). The collected soils were classified in three groups according to inherent characteristics (texture and calcareous properties). These groups were either i) relatively rich in clay and carbonates with high base saturation ("eutrophic'), ii) high in sand and relatively low pH and base saturation ("oligotrophic") or iii) intermediate fertility with siltier textures and intermediate base saturation ("mesotrophic"). We had applied these terms to denote the poorest soils ("oligotrophic") vs intermediately rich ("mesotrophic") and richest soils ("eutrophic") in our samples, and not to compare them outside our population (see Blondeel et al., 2019;Chapter 2).

To enable a comparison by land-use legacy, within each region, we searched for three adjacent pairs of ancient and post-agricultural broadleaved forest with similar canopy composition, leading to 48 forest patches in the study (Blondeel et al., 2019 and Chapter 2). We searched for pairs of forest patches with similar stand age, where one patch was in existence before 1850 (ancient forest) and the other established in mid-20th century (post-agricultural forest). We are consequently able to account for the effects of forest land-use legacies, i.e. alterations to soil biogeochemistry driven by past land use. Adjacent stand pairs had the same soil type classification in 21 out of 24 pairs, with the other three having a richer class on post-agricultural soil but similar classifications from the Soil World Reference Base (Chapter 2).

The legacies in the post-agricultural forest soils varied along gradients of inherent soil characteristics, regional P balance and N deposition (Blondeel et al., 2019 and Chapter 2). Overall, the soils from post-agricultural forest had 0.4 units higher pH (+0.41 ± 0.16 standard error (S.E.)) than in ancient forest (mean ancient pH = 4.82 ± 0.33 S.E.). Soil C:N was lower (-1.49 ± 0.24 S.E.) in post agricultural forest than in ancient forest (mean ancient C:N = 13.9 ± 0.94 S.E.). Finally, Soil P concentrations were elevetad in post agricultural forest for total P (+118 mg kg^{-1} ± 60 S.E.) and Olsen P (+13 mg kg^{-1} ± 5.5 S.E.) compared to ancient forest (mean ancient total P = 347 mg kg^{-1} ± 64.5 S.E.; mean ancient Olsen P = 15.3 mg kg^{-1} ± 4.5 S.E.).

We transported all 48 collected soil samples to our study site in the Aelmoeseneie forest (Belgium) during November 2015- February 2016. The sampled soil from each forest patch was divided over eight mesocosms, one for each two-level factorial combination of light x warming x temperature. Each mesocosm consisted of 13L sampled soil, placed in trays with 12 drainage holes (46.5 x 31.5 cm, depth of 19.5 cm) on top of 9 L of white sand to ensure proper drainage

(Figure 3.2). The bottom of the trays were first lined with a root fabric to ensure that plants would not root in the site's soil upon burying the mesocosms with their tops level to the ground.

Figure 3.2. Preparations for creating mesocosms. We filled 9 L of white sand on the bottom of the tray (front) before filling up the tray with of 13 L of soil sample (three trays in the back).

Plant community assembly: slow and fast colonisers grow together

We compiled a pool of fifteen species commonly found in temperate European forests (Table 3.1). Verheyen et al. (2003) quantified differences in functional and life-history traits among 216 herbaceous species that colonised and established in post-agricultural forests to varying degrees, allowing the determination of traits that lead to a successful colonisation. This meta-analysis was based on recorded observations of understorey species in either ancient forest or post-agricultural forest, across Western Europe. The varying success between species to colonise post-agricultural forest was captured in the colonisation capacity index (CCI). Larger negative values indicate a higher capacity to successfully colonise into post-agricultural forests

(Verheyen, Honnay, et al., 2003). The continuous CCI scale exists from -100 to +100, where -100 notes that the species was only found post-agricultural forest, while +100 means that the species remains confined within ancient forest. All but one species (*Hedera helix*) was reported by Verheyen et al. (2003), for this species we assume a CCI of 50 based on the fact that it can be dispersed by birds but that it has difficulty in colonising isolated forest patches (Butaye, Jacquemyn, & Hermy, 2001).

The three emergent groups comprise species that differ in their capacity to colonise post-agricultural forest and in forest habitat use. We based our classification on studies that had characterised typical ancient forest species in relation to a species' colonisation capacity (De Frenne, Baeten, et al., 2011; Verheyen, Honnay, et al., 2003) and habitat use as a forest specialist (Heinken et al., 2019). Taking the CCI and the classification by Heinken et al. (2019) together, the first group (A) are six poor colonisers and forest specialists, typical of ancient forest (Table 3.1). The second group (B) are six intermediate colonizers of post-agricultural forest and not strictly forest specialists (Supplementary Table 3.1). The final group (C) are three fast colonising and generalist nitrophilic species (Table 3.1). The classification is consequently not on the colonisation capacity index (CCI) alone. For those reasons, *Polygonatum multiflorum* is in group A as it is a typical forest species. Likewise, group B incorporates species with a wider range in habitat use and are not strictly forest specialists. *Ajuga reptans* and *Poa nemoralis* can occur in grassy habitats and grasslands, while *Hedera helix* is often found in shaded urban and rural habitats.

We assembled twelve communities (Table A3.1.1) in which we randomly assigned two poor colonisers (group A), two intermediate colonisers of post-agricultural forest (group B) and one fast-colonising nitrophilic species (group C). These twelve plant community combinations serve as representative assemblages in European mesotrophic deciduous forests. Each species occurs in four different assemblages. The twelve communities are repeated four times over the 48 soil samples in each two level factorial combination of light x warming x nitrogen addition (48 soil samples x 8 factorial combinations = 384 mesocosms). This repetition of communities was randomised using the *sample* function in R. Communities were allowed to differ on a given pair of a soil sample in a given treatment in the randomisation processes. This variation in communities on each soil increases generality of results given the trait-based focus of the study. However, this increasing generality trades-off with accuracy for estimating treatment effects, but is likely alleviated by the scale of study. The randomisation restricted the same species combination occurring multiple times on the same soil origin to avoid confounding of any soil type effect with a particular plant community.

Table 3.1. Pool of 15 species, in three groups based on prevalence in ancient forest, and their most important attributes. Scientific names are drawn from www.plantlist.org. Species are grouped in three emergent groups: slow colonisers and forest specialists (A), intermediate colonisers and not strictly forest specialists (B), and fast colonising generalist nitrophiles (C). We classified these species in three groups based on our expert knowledge, guided by the colonisation capacity index (CCI) and whether a species can be specified as a forest habitat specialist (Heinken et al., 2019). The CCI (Verheyen et al., 2003) ranges from 100 (slow colonisation into post-agricultural forest) to -100 (fast colonisation). Plant material used at the time of planting is indicated as well (March 2016). Symbols denote the origin of the plant material, and is either from an organic horticultural nursery (°, ECOFLORA, Belgium) or the native population in Brakelbos (*) and the Aelmoeseneie forest (**) in Belgium. The survival of a species after one growing season (2017) is listed as well, as the percentage of remaining individuals and the presence of a species in a mesocosm.

Scientific name	Group	Survival individual (max 512)	Survival mesocosm (max 128)	Life form	Peak biomass	CCI	Forest spec.?	Plant material
Hyacinthoides non-scripta (L.) Chouard ex Rothm	A	51%	78%	Geophyte	May	100	Yes	Bulb°
Galium odoratum (L.) Scop.	A	76%	80%	Hemicryptophyte	June	95	Yes	Whole plant°
Anemone nemorosa L.	A	28%	49%	Geophyte	April	77	Yes	Rhizome°
Carex sylvatica Huds	A	82%	89%	Therophyte	July-August	74	Yes	Whole plant°
Vinca minor L.	A	72%	85%	Chamaephyte	May-June	33	Yes	Whole plant°
Polygonatum multiflorum (L.) All.	A	75%	95%	Geophyte	May-June	15	Yes	Rhizome°
Ajuga reptans L.	B	19%	29%	Hemi cryptophyte	June	60	No	Whole plant°
Hedera helix L.	B	29%	52%	Chamaephyte	July-August	50	No	Whole plant°
Poa nemoralis L.	B	59%	75%	Hemicryptophyte	May-June	29	No	Whole plant*
Ficaria verna Huds.	B	85%	84%	Geophyte	March	11	No	Root nodule**
Glechoma hederacea L.	B	2%	3%	Hemicryptophyte	June	-5	No	Whole plant°
Geranium robertianum L.	B	10%	16%	Hemicryptophyte	June-July	-9	No	Whole plant°
Aegopodium podagraria L.	C	34%	55%	Hemicryptophyte	August-September	-45	No	Rhizome**
Poa trivialis L.	C	35%	50%	Hemicryptophyte	May-June	-67	No	Whole plant°
Urtica dioica L.	C	16%	37%	Hemicryptophyte	August-September	-71	No	Rhizome**
Total		45%	59%					

We planted the 384 mesocosms in the first week of March 2016. Each of the five species in a community were planted four times per mesocosm in a grid (7x8.5 cm) in a randomised order, resulting in 20 plants per mesocosm (Figure 3.3). We planted these species from vegetative plant material (Table 3.1) and obtained our plants from either the local population in the Aelmoeseneie forest or Brakelbos (Belgium), or a local plant nursery that deals in wild plants for horticulture (ECOFLORA, Halle, Belgium).

Figure 3.3. Example of one mesocosm (dimensions 31.5x46.5 cm) during the planting process. The auxiliary planting grid of 7x8.5 cm was used to plant the individuals in the correct place. Notice that the grids without aboveground biomass are not empty, but merely planted with rhizomes or bulbous plant material.

We buried all mesocosms with their tops at surface level, in the fenced area in the Aelmoeseneie Forest (Belgium) with a tree canopy (90- 95% cover) dominated by *Fagus sylvatica*, *Quercus robur, Acer pseudoplatanus*, *Fraxinus excelsior* and *Larix decidua* (Figure 3.4). We buried mesocosms in random groups of four ("Plot") to apply manipulative experimental treatments, as explained in the next section and following methods of De Frenne et al. (2015). In total, we planted 7680 (384 mesocosms x 20) individuals of fifteen species (512 individuals per species).

Manipulating the environment: N enrichment, experimental warming and enhanced light availability

We have applied three two-level treatments in a full-factorial design since April 2016 in the experimental area of the Aelmoeseneie forest (Figure 3.4). Ongoing treatments consist of (i) nitrogen enrichment; (ii) warming and (iii) enhanced light availability.

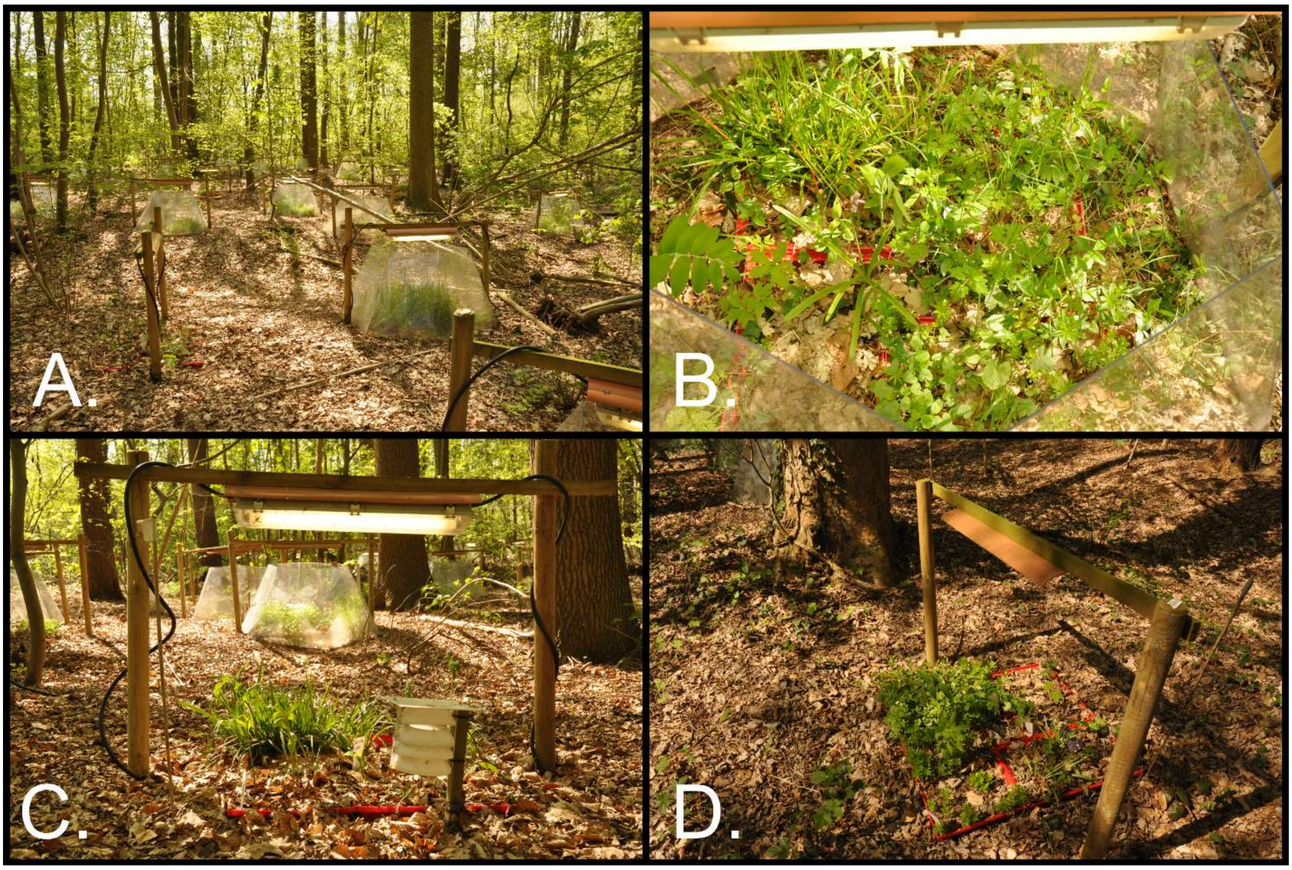


Figure 3.4. Images of the multifactor experiment, showing mesocosms in groups of four combined as a "plot". Panel A. Overall view of the fenced area in the Aelmoeseneie forest (Belgium). **Panel B**. A plot with a factorial combination of Warming and Light addition. **Panel C** Singular treatment of Light addition, with a temperature sensor to measure air temperature. **Panel D.** Control treatment where the plot receives ambient conditions. Plots that receive the N enrichment treatment are not discernible in the field.

The first treatment is N enrichment (further referred to as treatment "N") with an annual addition of 50 kg N ha^{-1} yr^{-1}. This treatment is performed by adding 0.25 L of a 2.01 g L-1 solution of NH_4NO_3 per mesocosm, which is applied four times per year (once each season) to reach a 50 kg N ha^{-1} yr^{-1} eq. in total (see Appendix section 3.2). During the addition, we rinsed the leaves with 0.25 L of demineralised water to ensure that the N fertiliser does not remain adhered to the vegetation surface. The controls receive ambient N deposition (15.5 kg N ha^{-1} yr^{-1}) and an additional volume of 0.5 L of demineralised water per seasonal addition.

The equivalent of 50 kg N ha^{-1} yr^{-1} on top of the ambient N deposition is a high N load, but not unusual near point sources of N deposition such as pig and cattle farms (Bobbink et al., 2015). We decided to apply N in its reactive form as NH_4^+ and NO_3^- to our mesocosms and not as NPK fertilizer in order isolate effects of reactive N on ecosystems (Stevens, 2019), as commonly performed in empirical ecology (Midolo et al., 2019). We did not take soil samples to check how the N treatment affected soil N availability, due to avoiding disturbance of the mesocosms. We could check the efficacy of the N addition treatment by measuring leaf N concentration of five abundant species across the whole experiment (Appendix 3.2). Of these five species, only

Ficaria verna had a significantly (p<0.05) higher leaf N concentration with N addition. The other four species (*Polygonatum multiflorum, Carex sylvatica, Poa nemoralis, Aegopodium podagraria*) did not respond in leaf N concentration to the N addition treatment. N limitation or N deficiency in the plots is consequently unlikely (see also Chapter 5).This shows that the N addition treatment only affected selected species, and probably not to a degree that can cause major shifts in community composition.

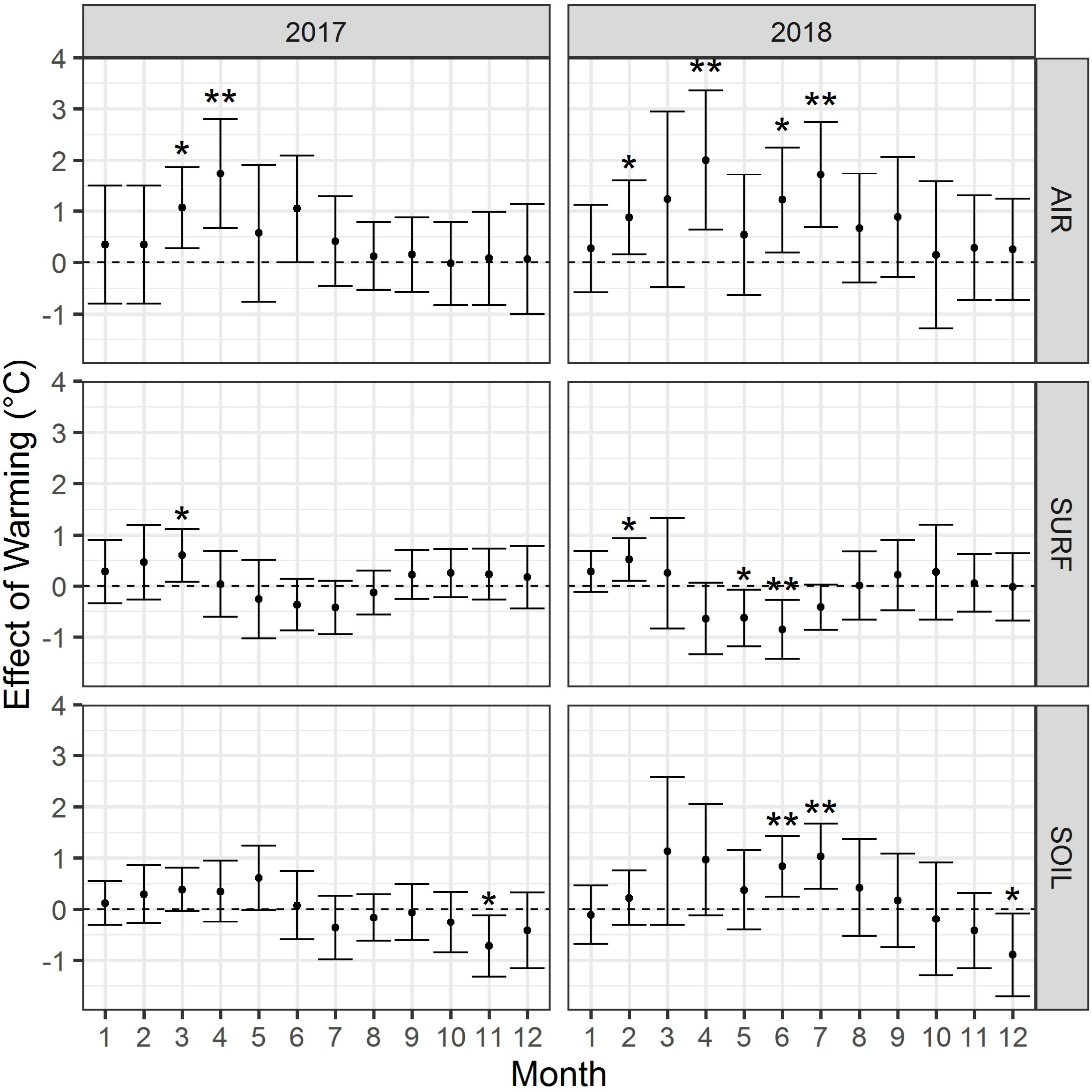


Figure 3.5. The warming treatment is most efficient in spring. Size of the warming effect, averaged over each Month in 2017 and 2018 at measured at three levels. We measured air temperatures (15 cm height), surface temperature (0 cm) and soil temperature (5 cm depth). Effects for which zero does not cross the 95% confidence intervals are marked with significance levels: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***). Spring and early summer show the largest increases in air temperatures, but differ by year. The surface cooling in summer 2018 (and tendency in summer 2017) could be due to evapotranspiration processes.

The second treatment consisted of experimental warming (referred to as treatment "T") with 75 cm-wide open top chambers, these are hexagonal greenhouse chambers with open tops (De Frenne et al., 2010, see Figure 3.4A-B). We measured air temperature (15 cm above forest floor), surface temperature (0 cm) and soil temperature (5 cm depth) in eight plots, one for each factorial combination of light x warming x nitrogen, using 24 thermocouples which log every 30 seconds (Type T miniature, TC Direct, The Netherlands and datalogger type CR1000, Campbell Scientific, USA). The warming effect of the open top chambers fluctuated throughout the two calendar years in which we recorded temperatures (2017 – 2018), with air temperature showing the largest warming effects, but less pronounced warming at surface level (0cm) and in the soil at 5 cm depth (Figure 3.5). The largest increases in monthly air temperatures occurred during April in both 2017 (+1.73 °C ± 0.54 S.E. and in 2018 (+2.00 °C ± 0.69 S.E). Springtime is optimal for passively warming the air temperatures with open top champers, as the forest canopy is not yet developed but solar radiation becomes more intense (De Frenne, Brunet, et al., 2011; De Frenne et al., 2010; Pelini et al., 2011). We decided to apply passive warming with open top chambers due to its low cost and energy inputs, favouring this method over active warming with infrared-heaters (Aronson & Mcnulty, 2009; Kimball et al., 2015; Yang et al., 2018). Furthermore, potential artefacts of open top chambers on wind speed and precipitation patterns are generally limited in forest ecosystems due to the buffering role of the canopy and shrub layer (De Frenne et al., 2010). We did not find any open top chamber effects on soil moisture content (Table A3.4.1) or herbivore dynamics (see Box 3.1).

The third treatment (Figure 3.4B-C), light addition (referred to as "L"), adds 23.98±4.40 μmol $m^{-2}s^{-1}$ PAR to the ambient light conditions (7.79±0.68 μmol $m^{-2}s^{-1}$ under fully closed canopy, see Figure 3.6). We installed two 18 W fluorescent tubes suspended 75 cm above ground level of each treated plot. These lights are programmed to follow the natural photoperiod throughout the year and do not significantly affect air temperatures (De Frenne et al., 2015). Control plots (Figure 3.4D) receive ambient light and have a dummy lamppost suspended over the plot to account for undesired side effects of the lamp installation other than light addition (e.g. precipitation). This lamp installation is effective in forests (De Frenne et al., 2015), as the relationship between PAR and mean carbon assimilation rates is strongly non-linear. There is a light saturation point at around 500 μmol $m^{-2}s^{-1}$ with the largest increment in carbon assimilation before 100 μmol $m^{-2}s^{-1}$ in typical forest understorey plants (Rothstein & Zak, 2001). The light treatment could thus more than double the mean CO_2 assimilation rates in our test plants (Rothstein & Zak, 2001) by adding 23.98±4.40 μmol $m^{-2}s^{-1}$ PAR to the low ambient light conditions (7.79±0.68 μmol $m^{-2}s^{-1}$) under the closed canopy (Figure 3.6).

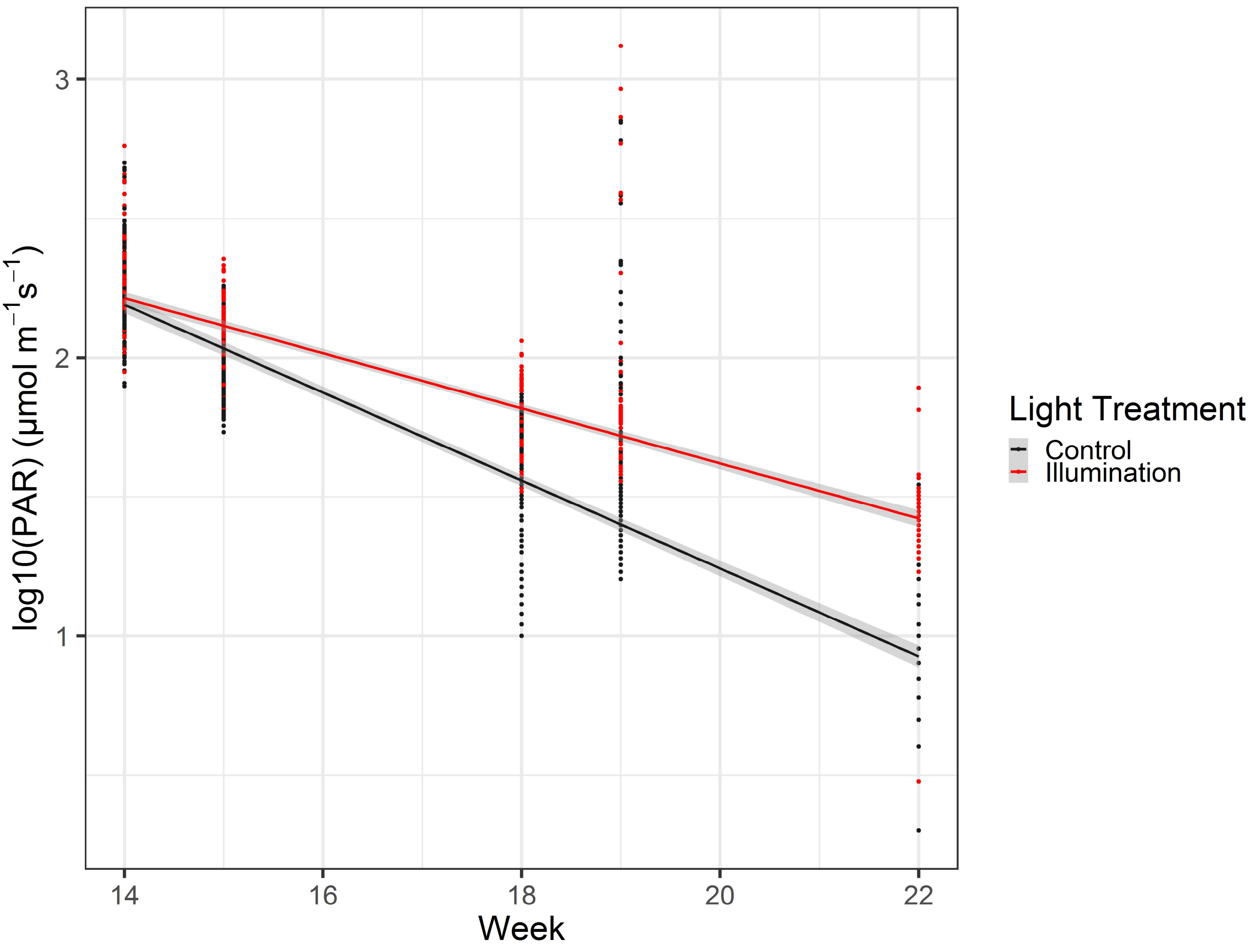


Figure 3.6. Subcanopy photosynthetically active radiation (PAR) decreases towards late spring, while the light availability treatment increases PAR. These measurements were performed on 7 April 2017 (partially Cloudy), 11 April 2017 (cloudy), 14 April 2017 (cloudy), 5 May 2017 (cloudy), 10 May 2017 (sunny) and 1 June 2016 (partially cloudy). Measurement conditions varied from cloudy to sunny, with the outliers likely being sunflecks. The illumination treatment adds 23.98±4.40 μmol/m^{2}s PAR to the ambient light conditions. Later in spring with a dense canopy (1 June 2016), mean ambient PAR (control) was 7.79±0.68 μmol/m^{2}s. The vertical axis is in a base 10 logarithmical scale to show the difference in low PAR values (around 10 μmol/m^{2}s) more clear.

Summarising the design of the experiment (Figure 3.7, Figure A3.3.1, Table A3.3.1), there are eight regional origins of soil, with three ancient and three post-agricultural forest soils (LU) from each region (48 soil samples). These 48 soil samples were divided in eight equal portions and randomly planted with one of 12 plant communities (5 species per community, 20 individuals in total) to create “mesocosms”. These mesocosms (384) were exposed to two-level treatments of N enrichment (N), warming (T) and light (L) addition (8 combinations).

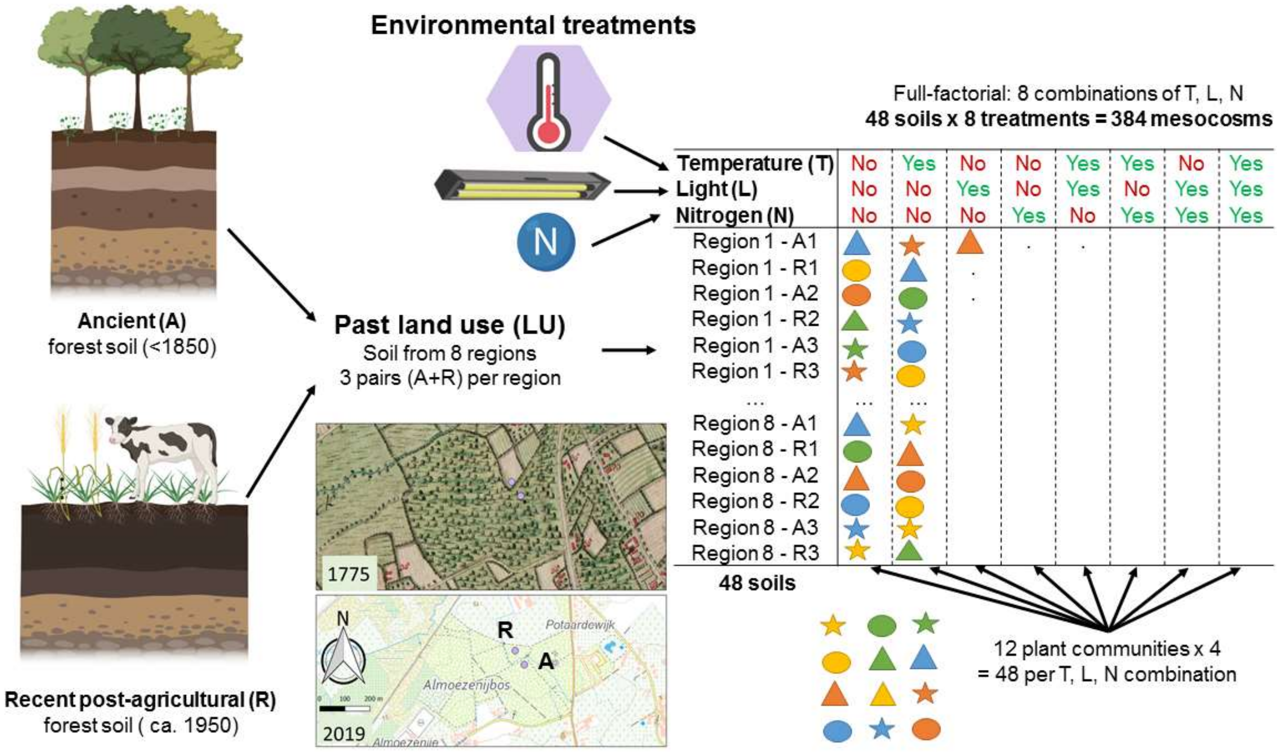


Figure 3.7. Schematic overview of the main features of the experimental design. The land-use history of the forest soil (LU) is the main factor in this design. There are 48 soil origins in this experiment, coming from eight different European regions. These soils are from three paired forest patches per region, one ancient forest and one post-agricultural forest per pair. We classified these soils post-hoc into three soil groups based on inherent soil fertility (see Chapter 2, not in figure). These 48 soil samples were equally distributed over the full-factorial combinations (8) of the two-level treatments for temperature (T), light addition (L) and N addition (N). We randomy assembled 12 plant communities, which consist of five species (20 individuals) with different colonisation capacity and affinity to forest in each community assembled from a pool of 15 species. These twelve communities are repeated four times over the 48 soil samples in each two level factorial combination of T x L x N addition (48 soil samples x 8 factorial combinations = 384 mesocosms).

We additionally checked in all mesocosms whether there were unwanted treatment side-effects of treatments on soil moisture availability (Appendix 3.4). Treatments of warming ($p = 0.67$), light addition ($p = 0.27$) and N addition (0.26) did not significantly affect soil moisture availability (Appendix Table A3.4.1). The soil moisture, however, varied between soil types and with the forest land-use history. Mesotrophic soils had the highest soil moisture content ($p < 0.001$), as did ancient forest soils ($p<0.001$). The higher water retention in ancient forest soils could be due to a larger C:N ratio (Chapter 2) compared to post-agricultural forest (Verry et al., 2011).

Plant survival

We counted individual survival and species survival in the mesocosms one year after initial planting (2017). During the initial planting season (2016), we scored individuals on mortality in the first week of May in order to replant individuals when they were either dead or missing (see Figure A3.5.1). Species that multiply using rhizomes or bulbs (e.g. *Anemone nemorosa* or *Hyacinthoides non-scripta*) were replanted when there was no sign of aboveground biomass or bud emergence. *Poa* grasses were replanted in September (to aid identification) during a weeding process that occurred in all mesocosms (Figure A3.5.2). The replanting of individuals only occurred once and was to allow a sufficient amount of test species to persist for community development and further analysis.

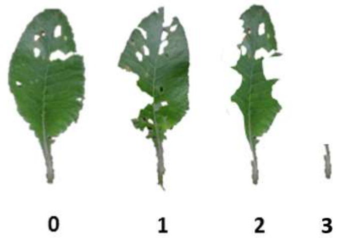
After one year, 3445 of the 7680 planted individuals survived (45%). These individual counts were made at the time of each species' peak biomass (Table 3.1) and translate into the number of plant height measurements (further details see Chapter 4). The number of plant height measurements (i.e. individual survivors) did not depend on treatment effects (see Figure A4.1.1). The survival of species on the individual level varied widely between species (Table 3.1). The most persistent individuals were found in *Ficaria verna*, with 85% of the individuals surviving the next year. The least persistent species was *Glechoma hederacea,* with merely 12 individual survivors (2 %). When counting the presence of a species in a mesocosm, we found that *Carex sylvatica* persisted in 89% of the mesocosms while *G. hederacea* remained the poorest survivor with a persistence in 3% of the mesocosms (Table 3.1). A main driver for the loss of *G. hederacea* is herbivory by slugs (see Box 3.1).

Conclusions

This methodological chapter laid out the experimental design that provides the foundation for further research questions and analysis. The methods, tables and illustrations presented here are thus a vehicle to contextualize further experimental methods and results presented in Chapters 4 – 6. In summary, we bypassed dispersal limitation in species by planting slow and fast colonisers on soils from both ancient and post-agricultural forest ("mesocosms"). This planting scheme separates the dispersal limitation in species from the agricultural legacy, so that we can focus on the biogeochemical aspect of agricultural land-use legacy effects. We applied experimental treatments of warming, N addition and enhanced light availability to these mesocosm in a full-factorial approach. This full-factorial approach disentangles these common environmental drivers in forests. For instance, N availability and temperatures commonly increase with bright conditions induced by a forest gap. Our experiment changes each driver separately, helping to unravel the mechanism underlying responses observed in gaps. This full-factorial design provides the foundation for the coming chapters, where we explore plant trait responses to the global change treatments, scaling up from within-species responses in traits (Chapter 4) and nutrient uptake (Chapter 5) to community change in traits over time (Chapter 6).

Box 3.1. The red slug (*Arion rufus*) drives initial mortality in ground-ivy (*Glechoma hederacea*)

While replanting species in May 2016, we found a high level of red slug attack on *Glechoma hederacea***.** We then decided to record herbivory in all mesocosms in July and September. Herbivore damage on leaves was assessed using a simple scoring system (Figure B3.1.1) following methods of de la Peña *et al.* (2016). These authors found significantly more traces of herbivory on experimental plant populations on post-agricultural forest soils, which could be attributed to a higher plant nutrient content (more specifically P). We expected a similar response in our experiment, which could be aggravated by the nitrogen addition treatment. However, ANOVA on community weighted mean herbivory in the mesocosms showed that the anticipated interaction between N addition on post-agricultural soil did not occur (p = 0.15). In addition, warming did not affect herbivory (p = 0.55), nor did light addition (p = 0.42), N addition (p = 0.89) or the forest land-use history (p = 0.69) as main effects.



0. Negligible herbivory. Less than 5% of leaf surface removed
1. Moderate herbivory. 5 – 30% of leaf surface removed
2. Severe herbivory. 30-70% of leaf surface removed.
3. (Almost) complete defoliation. 70-100% of leaf surface

Figure B3.1.1. Ordinal herbivory score, adapted from de la Peña et al. (2016).

Our initial field observation (May 2016) of the attack on *Glechoma hederacea* was visible in the herbivory score of this species that we recorded later in that growing season (Figure B3.1.2). The replanted individuals had been largely eaten by July, with still visible marks of herbivory on leaves. By September that year, nearly all individuals were gone, and only those with negligible herbivory survived. The persistence of *Glechoma hederacea* into the next growing season was thus extremely low (2 %) due to the palatability of this species.

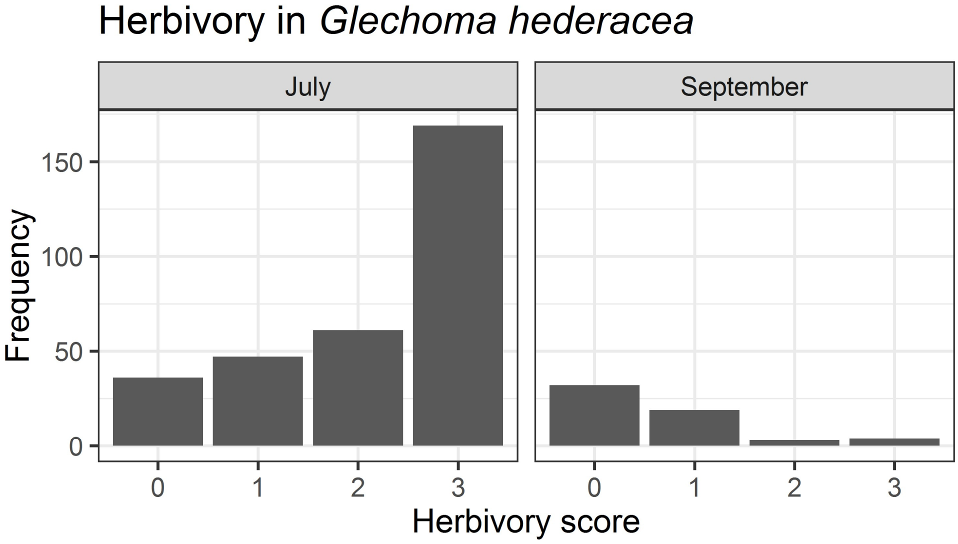


Figure B3.1.2. Frequency of herbivory scores in *Glechoma hederacea*. Note that frequencies of this species dropped markedly in September as it has been heavily attacked two months prior.

Appendix 3.1. Community assembly

Table A3.1.1. Species combinations in the twelve communities. The combinations always have two slow colonisers and strictly forest specialists (group A), two intermediate colonisers and species regularly found in post-agricultural forest but not strictly forest specialist (group B) and one fast colonising nitrophilous species (group C). These species were randomised into these 12 combinations using the "sample" function in R. Each species combination is repeated over 32 mesocosms.

Com.	**Group A – Slow colonisers**		**Group B – intermediate colonisers**		**Group C - Nitrophiles**
com1	*G. odoratum*	*P. multiflorum*	*A. reptans*	*H. helix*	*P. trivialis*
com2	*C. sylvatica*	*H. non-scripta*	*G. hederacea*	*A. reptans*	*A. podagraria*
com3	*H. non-scripta*	*A. nemorosa*	*P. nemoralis*	*G. hederacea*	*A. podagraria*
com4	*C. sylvatica*	*P. multiflorum*	*A. reptans*	*F. verna*	*P. trivialis*
com5	*C. sylvatica*	*P. multiflorum*	*G. robertianum*	*H. helix*	*A. podagraria*
com6	*A. nemorosa*	*V. minor*	*P. nemoralis*	*G. robertianum*	*P. trivialis*
com7	*H. non-scripta*	*C.sylvatica*	*H. helix*	*G. hederacea*	*U. dioica*
com8	*V. minor*	*G. odoratum*	*F. verna*	*A. reptans*	*A. podagraria*
com9	*A. nemorosa*	*H. non-scripta*	*G. robertianum*	*P. nemoralis*	*P. trivialis*
com10	*P. multiflorum*	*V. minor*	*G. robertianum*	*F. verna*	*U. dioica*
com11	*V. minor*	*G. odoratum*	*P. nemoralis*	*H. helix*	*U. dioica*
com12	*A. nemorosa*	*G. odoratum*	*G. hederacea*	*F. verna*	*U. dioica*

Appendix 3.2. N treatment details

Required amount of NH_4NO_3 in N addition treatment

Target: 50 k$g\ N\ ha^{-2}\ year^{-1}$= 5 $g\ N\ m^{-2}\ year^{-1}$

Molar mass of $N$is 14.0067 g mol^{-1}, the amount of Namount per m^2:

$$\frac{5\ g\ N\ m^{-2}\ year^{-1}}{14.0067\ \ g\ mol^{-1}} = 0.357\ mol\ N\ m^{-2} year^{-1}$$

N is applied as NH_4NO_3 (molar mass = 80.04336 g mol^{-1}):

$$\frac{2\ mol\ N}{80.04336\ g\ NH_4NO_3} = 0.025\ mol\ N\ g^{-1}\ NH_4NO_3$$

Mass (g) of NH_4NO_3 per m^2:

$$\frac{0.357\ mol\ N\ m^{-2} year^{-1}}{0.025\ mol\ N\ g^{-1}\ NH_4NO_3} = 14.28\ g\ NH_4NO_3\ m^{-2} year^{-1}$$

The NH_4NO_3 fertiliser is added per plot (group of four mesocosms). Each mesocosm is 0.315 m x 0.465 m in surface area, with a centre unused area in the plot of 0.15 m x 0.15 m. The area in consideration is thus:

$$4 * (0.315 * 0.465)\ m^2 - (0.15^2)\ m^2 = 0.563\ \ m^2$$

Amount of NH_4NO_3 added to this plot area (4 mesocosms) in each year:

$$14.28\ g\ NH_4NO_3\ m^{-2} year^{-1} * 0.563 = 8.04\ g\ NH_4NO_3\ year^{-1}$$

The addition of NH_4NO_3 occurs seasonally (4 times per year):

$$\frac{8.04\ g\ NH_4NO_3\ year^{-1}}{4} = 2.01\ g\ NH_4NO_3\ per\ season$$

Each season, this amount of 2.01 g NH_4NO_3 is delivered in a 1 L solution and applied evenly across the four mesocosms in a Plot (0.25 L per mesocosm).

To back-check this calculation, each mesocosm receives per addition:

$$0.25\ L * 2.01\ g\ L^{-1}\ NH_4NO_3\ =\ 0.5025 g\ NH_4NO_3$$

For the surface area of 0.140875 m^2 (Plot area of 0.563 m^2 divided by 4), this becomes:

$$\frac{0.5025\ g\ NH_4NO_3}{0.140875\ m^2} = 3.567\ g\ NH_4NO_3\ \ m^{-2}\ or\ 35.67\ kg\ NH_4NO_3\ \ ha^{-1}$$

N weight in NH_4NO_3 is 35%:

$$0.35 * 35.67\ kg\ NH_4NO_3\ \ ha^{-1} = 12.48\ kg\ N\ ha^{-1}$$

Which is applied four times per year:

$$12.48\ kg\ N\ ha^{-1} * 4 = 49.92\ kg\ N\ ha^{-1} = 50\ kg\ N\ ha^{-1}$$

Efficacy of N treatment: does leaf N concentration of species change?

During the collection of leaf samples for SLA (Chapter 4), we selected 5 abundant species to estimate changes in leaf N due to the N addition treatment (Table A3.2.1). Leaf samples were weighed (dry mass) and ground after drying to constant weight at 70°C for 48 h. We selected species for which we had enough mass remaining after drying to perform analysis on total leaf N concentration (30 mg). These five species have a distribution that is akin to the species combinations for the plant communities (2 group A, 2 group B, 1 group C). We measured total N concentration (%) by high temperature combustion at 1150°C using an elemental analyzer (Vario MACRO cube CNS, Elementar, Germany).Of these species, only *Ficaria verna* showed a significantly higher leaf N concentration (+0.37 ± 0.09, p = 0.0001). Other than that, no significant changes in leaf N concentration were found (Table A3.2.2). The influence of the N addition treatment on plant N uptake thus remains ambiguous and individualistic within plant species.

Table A3.2.1. Number of leaf samples for leaf N according to the N addition treatment. The maximal number of leaf samples per species is 64 for control, and 64 for the treatment (128 in total).

Species	Group	N treatment	number
Polygonatum multiflorum	A	Control	61
Polygonatum multiflorum	A	Treatment	61
Carex sylvatica	A	Control	59
Carex sylvatica	A	Treatment	55
Ficaria verna	B	Control	55
Ficaria verna	B	Treatment	53
Poa nemoralis	B	Control	49
Poa nemoralis	B	Treatment	47
Aegopodium podagraria	C	Control	32
Aegopodium podagraria	C	Treatment	39

Table A3.2.2. Estimates of leaf N concentration (%) for 5 selected species in response to N addition.

Species	Group	term	estimate	S.E.	t-statistic	p-value
Polygonatum multiflorum	A	(Intercept)	3.56	0.09	40.1	<0.0001
Polygonatum multiflorum	A	N treatment	-0.05	0.13	-0.397	0.69
Carex sylvatica	A	(Intercept)	2.89	0.08	36.68	<0.0001
Carex sylvatica	A	N treatment	-0.11	0.11	-0.96	0.34
Ficaria verna	B	(Intercept)	3.75	0.07	52.37	<0.0001
Ficaria verna	B	N treatment	0.37	0.09	4.04	0.0001
Poa nemoralis	B	(Intercept)	4.14	0.17	23.71	<0.0001
Poa nemoralis	B	N treatment	0.32	0.25	1.31	0.20
Aegopodium podagraria	C	(Intercept)	3.94	0.11	35.04	<0.0001
Aegopodium podagraria	C	N treatment	-0.04	0.15	-0.27	0.79

Appendix 3.3. Experimental design overview

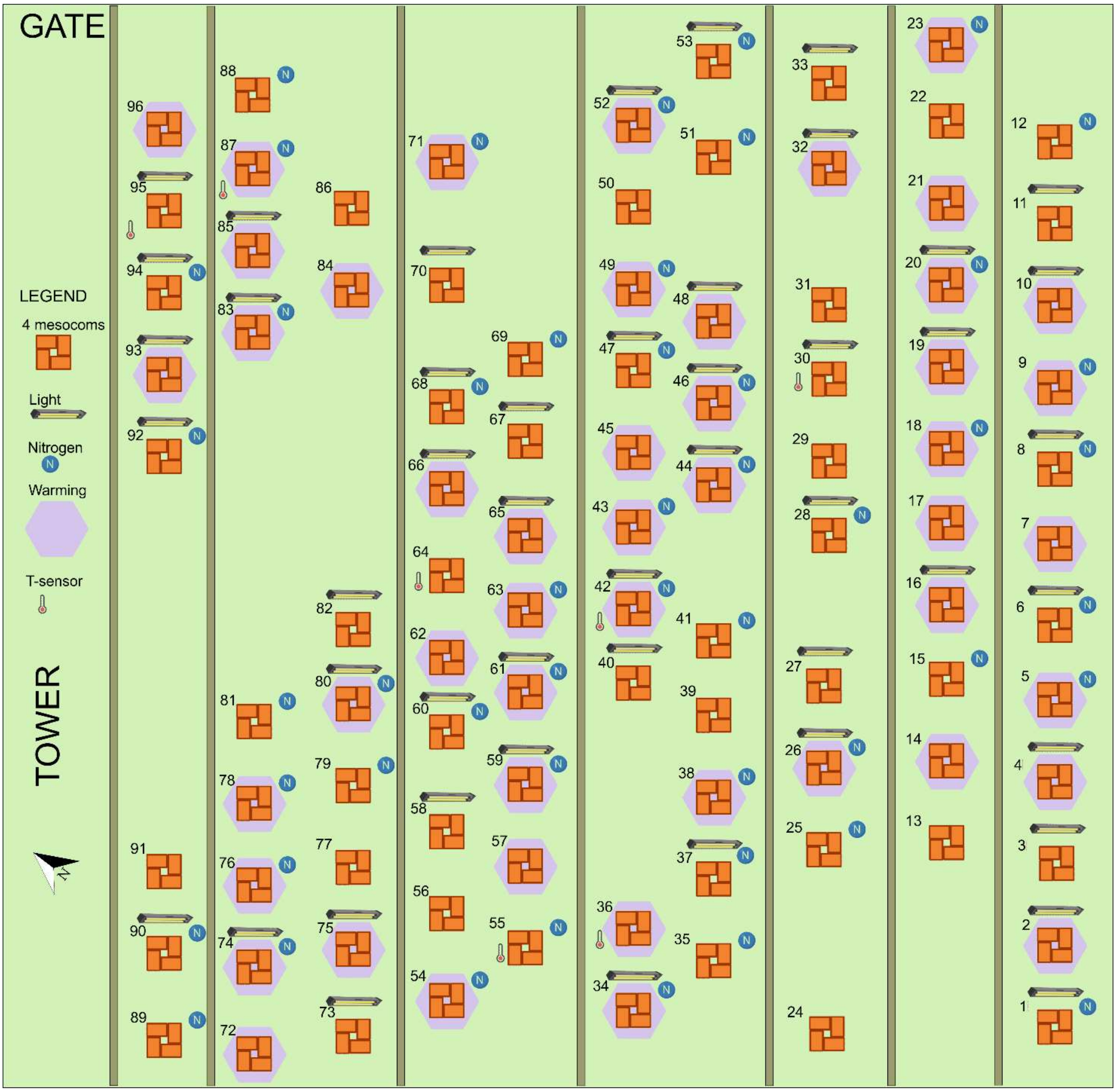


Figure A3.3.1. Schematic map of the field experiment in the forest, with all mesocosms organized in "Plots" of four. Each mesocosm is here represented as a red rectangle, which grouped by four create a composite square (with a 15 x 15 cm^2 gap in the middle). The specific details for each plot and mesocosm are provided in Table A3.3.1, including the the land-use history of the soils for each mesocosm. Light addition, warming and N addition are noted in the image with designated icons. Plots for temperature monitoring are also noted with a designated icon. Plot numbers are provided in the image, these can be linked to Table A3.3.1 for further details on the mesocosms. The "empty" area's in the schematic map note that there is a small canopy gap in that area, so that no plot was placed there. The vertical lines in the image are "rabatten", i.e. drainage ditches that are common in ancient forests across Flanders. The plots were always established on the ridges of these soil heaps, and never in the drainage ditch itself. For orientation, a north arrow and the position of the measuring tower (left) and entry gate to the site (top left) are provided.

Table A3.3.1. All 384 mesocosms and their most important characteristics. The plot number is the random group of four mesocosms. Each mesocosm has a unique number and a community (one of twelve, see Table A3.1.1) assigned to it. The soil sample shows the regional origin (8 regions, abbreviations see Blondeel et al. 2019 and Chapter 2), a code noting the land-use history (with two levels, "Ancient" (A, in existence pre 1950) and recent post-agricultural (R, in existence since 1950)), and the pair of the sample. Land-use history is also given in a separate column. The treatment abbreviations: C (control), T (warming), L (light), N (nitrogen addition), TL (warming and light), TN (warming and nitrogen), LN (light and nitrogen), TLN (warming, light and nitrogen). Soil types are oligotriphic ("Oligo"), mesotrophic ("Meso") and eutrophic ("Eu") (see Blondeel et al 2019 and Chapter 2).

Plot	Mesocosm	Community	Sample	Region	Land-use history	Treatment	Soil type
1	103	com12	EST-A1	EST	Ancient	LN	Meso
1	111	com12	EST-R1	EST	Post-agricultural	LN	Meso
1	191	com2	PR-R3	PR	Post-agricultural	LN	Oligo
1	223	com11	BR-R2	BR	Post-agricultural	LN	Oligo
2	53	com4	SW-A1	SW	Ancient	TL	Oligo
2	253	com9	AM-R1	AM	Post-agricultural	TL	Eu
2	317	com6	OR-R2	OR	Post-agricultural	TL	Oligo
2	325	com3	OR-A3	OR	Ancient	TL	Meso
3	11	com4	VL-R1	VL	Post-agricultural	L	Meso
3	163	com9	PR-A2	PR	Ancient	L	Oligo
3	171	com10	PR-R2	PR	Post-agricultural	L	Oligo
3	299	com3	OR-R1	OR	Post-agricultural	L	Oligo
4	189	com12	PR-R3	PR	Post-agricultural	TL	Oligo
4	213	com9	BR-A2	BR	Ancient	TL	Oligo
4	261	com9	AM-A2	AM	Ancient	TL	Meso
4	285	com10	AM-R3	AM	Post-agricultural	TL	Meso
5	46	com11	VL-R3	VL	Post-agricultural	TN	Meso
5	62	com9	SW-R1	SW	Post-agricultural	TN	Oligo
5	150	com1	PR-A1	PR	Ancient	TN	Oligo
5	342	com5	CH-A1	CH	Ancient	TN	Eu
6	175	com2	PR-R2	PR	Post-agricultural	LN	Oligo
6	199	com6	BR-A1	BR	Ancient	LN	Oligo
6	255	com5	AM-R1	AM	Post-agricultural	LN	Eu
6	279	com9	AM-A3	AM	Ancient	LN	Meso
7	50	com11	SW-A1	SW	Ancient	T	Oligo
7	82	com9	SW-A3	SW	Ancient	T	Oligo
7	114	com5	EST-A2	EST	Ancient	T	Oligo
7	306	com5	OR-A2	OR	Ancient	T	Oligo
8	55	com10	SW-A1	SW	Ancient	LN	Oligo
8	95	com7	SW-R3	SW	Post-agricultural	LN	Meso
8	143	com8	EST-R3	EST	Post-agricultural	LN	Oligo
8	215	com7	BR-A2	BR	Ancient	LN	Oligo
9	38	com11	VL-A3	VL	Ancient	TN	Meso
9	190	com1	PR-R3	PR	Post-agricultural	TN	Oligo
9	198	com5	BR-A1	BR	Ancient	TN	Oligo
9	302	com1	OR-R1	OR	Post-agricultural	TN	Oligo
10	21	com11	VL-A2	VL	Ancient	TL	Meso
10	37	com6	VL-A3	VL	Ancient	TL	Meso
10	85	com11	SW-A3	SW	Ancient	TL	Oligo
10	173	com5	PR-R2	PR	Post-agricultural	TL	Oligo
11	3	com11	VL-A1	VL	Ancient	L	Meso
11	227	com7	BR-A3	BR	Ancient	L	Oligo
11	267	com8	AM-R2	AM	Post-agricultural	L	Eu
11	379	com5	CH-R3	CH	Post-agricultural	L	Eu
12	52	com9	SW-A1	SW	Ancient	N	Oligo
12	284	com7	AM-R3	AM	Post-agricultural	N	Meso
12	372	com4	CH-A3	CH	Ancient	N	Eu
12	380	com7	CH-R3	CH	Post-agricultural	N	Eu
13	9	com8	VL-R1	VL	Post-agricultural	C	Meso
13	137	com5	EST-R3	EST	Post-agricultural	C	Oligo
13	225	com10	BR-A3	BR	Ancient	C	Oligo
13	273	com3	AM-A3	AM	Ancient	C	Meso
14	58	com4	SW-R1	SW	Post-agricultural	T	Oligo
14	138	com7	EST-R3	EST	Post-agricultural	T	Oligo
14	154	com12	PR-R1	PR	Post-agricultural	T	Oligo
14	186	com3	PR-R3	PR	Post-agricultural	T	Oligo
15	76	com5	SW-R2	SW	Post-agricultural	N	Meso

Plot	Mesocosm	Community	Sample	Region	Land-use history	Treatment	Soil type
15	116	com11	EST-A2	EST	Ancient	N	Oligo
15	132	com11	EST-A3	EST	Ancient	N	Oligo
15	212	com3	BR-A2	BR	Ancient	N	Oligo
16	133	com8	EST-A3	EST	Ancient	TL	Oligo
16	141	com11	EST-R3	EST	Post-agricultural	TL	Oligo
16	181	com6	PR-A3	PR	Ancient	TL	Oligo
16	277	com1	AM-A3	AM	Ancient	TL	Meso
17	218	com1	BR-R2	BR	Post-agricultural	T	Oligo
17	258	com5	AM-A2	AM	Ancient	T	Meso
17	282	com5	AM-R3	AM	Post-agricultural	T	Meso
17	314	com12	OR-R2	OR	Post-agricultural	T	Oligo
18	78	com2	SW-R2	SW	Post-agricultural	TN	Meso
18	134	com5	EST-A3	EST	Ancient	TN	Oligo
18	310	com9	OR-A2	OR	Ancient	TN	Oligo
18	318	com11	OR-R2	OR	Post-agricultural	TN	Oligo
19	157	com1	PR-R1	PR	Post-agricultural	TL	Oligo
19	229	com11	BR-A3	BR	Ancient	TL	Oligo
19	309	com2	OR-A2	OR	Ancient	TL	Oligo
19	365	com8	CH-R2	CH	Post-agricultural	TL	Eu
20	104	com1	EST-A1	EST	Ancient	TLN	Meso
20	160	com10	PR-R1	PR	Post-agricultural	TLN	Oligo
20	200	com1	BR-A1	BR	Ancient	TLN	Oligo
20	248	com11	AM-A1	AM	Ancient	TLN	Meso
21	42	com8	VL-R3	VL	Post-agricultural	T	Meso
21	98	com2	EST-A1	EST	Ancient	T	Meso
21	210	com10	BR-A2	BR	Ancient	T	Oligo
21	346	com4	CH-R1	CH	Post-agricultural	T	Eu
22	113	com1	EST-A2	EST	Ancient	C	Oligo
22	217	com3	BR-R2	BR	Post-agricultural	C	Oligo
22	257	com8	AM-A2	AM	Ancient	C	Meso
22	321	com6	OR-A3	OR	Ancient	C	Meso
23	14	com10	VL-R1	VL	Post-agricultural	TN	Meso
23	182	com1	PR-A3	PR	Ancient	TN	Oligo
23	270	com3	AM-R2	AM	Post-agricultural	TN	Eu
23	382	com10	CH-R3	CH	Post-agricultural	TN	Eu
24	57	com5	SW-R1	SW	Post-agricultural	C	Oligo
24	65	com1	SW-A2	SW	Ancient	C	Meso
24	97	com5	EST-A1	EST	Ancient	C	Meso
24	241	com12	AM-A1	AM	Ancient	C	Meso
25	92	com10	SW-R3	SW	Post-agricultural	N	Meso
25	108	com8	EST-R1	EST	Post-agricultural	N	Meso
25	148	com9	PR-A1	PR	Ancient	N	Oligo
25	332	com4	OR-R3	OR	Post-agricultural	N	Meso
26	24	com12	VL-A2	VL	Ancient	TLN	Meso
26	64	com2	SW-R1	SW	Post-agricultural	TLN	Oligo
26	72	com3	SW-A2	SW	Ancient	TLN	Meso
26	128	com3	EST-R2	EST	Post-agricultural	TLN	Oligo
27	59	com12	SW-R1	SW	Post-agricultural	L	Oligo
27	67	com8	SW-A2	SW	Ancient	L	Meso
27	243	com9	AM-A1	AM	Ancient	L	Meso
27	275	com2	AM-A3	AM	Ancient	L	Meso
28	15	com1	VL-R1	VL	Post-agricultural	LN	Meso
28	79	com8	SW-R2	SW	Post-agricultural	LN	Meso
28	311	com11	OR-A2	OR	Ancient	LN	Oligo
28	367	com11	CH-R2	CH	Post-agricultural	LN	Eu
29	33	com8	VL-A3	VL	Ancient	C	Meso
29	49	com6	SW-A1	SW	Ancient	C	Oligo
29	145	com10	PR-A1	PR	Ancient	C	Oligo
29	249	com2	AM-R1	AM	Post-agricultural	C	Eu
30	43	com4	VL-R3	VL	Post-agricultural	L	Meso
30	155	com2	PR-R1	PR	Post-agricultural	L	Oligo
30	187	com10	PR-R3	PR	Post-agricultural	L	Oligo
30	211	com4	BR-A2	BR	Ancient	L	Oligo
31	161	com7	PR-A2	PR	Ancient	C	Oligo
31	313	com9	OR-R2	OR	Post-agricultural	C	Oligo
31	329	com7	OR-R3	OR	Post-agricultural	C	Meso
31	361	com7	CH-R2	CH	Post-agricultural	C	Eu
32	77	com4	SW-R2	SW	Post-agricultural	TL	Meso
32	109	com5	EST-R1	EST	Post-agricultural	TL	Meso

Plot	Mesocosm	Community	Sample	Region	Land-use history	Treatment	Soil type
32	293	com6	OR-A1	OR	Ancient	TL	Oligo
32	333	com3	OR-R3	OR	Post-agricultural	TL	Meso
33	123	com5	EST-R2	EST	Post-agricultural	L	Oligo
33	131	com2	EST-A3	EST	Ancient	L	Oligo
33	283	com2	AM-R3	AM	Post-agricultural	L	Meso
33	315	com1	OR-R2	OR	Post-agricultural	L	Oligo
34	152	com7	PR-A1	PR	Ancient	TLN	Oligo
34	216	com2	BR-A2	BR	Ancient	TLN	Oligo
34	280	com8	AM-A3	AM	Ancient	TLN	Meso
34	336	com9	OR-R3	OR	Post-agricultural	TLN	Meso
35	140	com9	EST-R3	EST	Post-agricultural	N	Oligo
35	204	com12	BR-R1	BR	Post-agricultural	N	Oligo
35	276	com12	AM-A3	AM	Ancient	N	Meso
35	340	com1	CH-A1	CH	Ancient	N	Eu
36	18	com2	VL-A2	VL	Ancient	T	Meso
36	26	com1	VL-R2	VL	Post-agricultural	T	Meso
36	122	com6	EST-R2	EST	Post-agricultural	T	Oligo
36	338	com3	CH-A1	CH	Ancient	T	Eu
37	7	com5	VL-A1	VL	Ancient	LN	Meso
37	31	com6	VL-R2	VL	Post-agricultural	LN	Meso
37	271	com12	AM-R2	AM	Post-agricultural	LN	Eu
37	343	com7	CH-A1	CH	Ancient	LN	Eu
38	6	com12	VL-A1	VL	Ancient	TN	Meso
38	54	com8	SW-A1	SW	Ancient	TN	Oligo
38	118	com7	EST-A2	EST	Ancient	TN	Oligo
38	222	com8	BR-R2	BR	Post-agricultural	TN	Oligo
39	129	com12	EST-A3	EST	Ancient	C	Oligo
39	153	com11	PR-R1	PR	Post-agricultural	C	Oligo
39	193	com10	BR-A1	BR	Ancient	C	Oligo
39	305	com12	OR-A2	OR	Ancient	C	Oligo
40	195	com9	BR-A1	BR	Ancient	L	Oligo
40	203	com5	BR-R1	BR	Post-agricultural	L	Oligo
40	323	com12	OR-A3	OR	Ancient	L	Meso
40	339	com11	CH-A1	CH	Ancient	L	Eu
41	20	com10	VL-A2	VL	Ancient	N	Meso
41	252	com1	AM-R1	AM	Post-agricultural	N	Eu
41	268	com2	AM-R2	AM	Post-agricultural	N	Eu
41	308	com4	OR-A2	OR	Ancient	N	Oligo
42	8	com4	VL-A1	VL	Ancient	TLN	Meso
42	144	com10	EST-R3	EST	Post-agricultural	TLN	Oligo
42	288	com8	AM-R3	AM	Post-agricultural	TLN	Meso
42	368	com5	CH-R2	CH	Post-agricultural	TLN	Eu
43	30	com7	VL-R2	VL	Post-agricultural	TN	Meso
43	166	com4	PR-A2	PR	Ancient	TN	Oligo
43	206	com10	BR-R1	BR	Post-agricultural	TN	Oligo
43	238	com4	BR-R3	BR	Post-agricultural	TN	Oligo
44	40	com4	VL-A3	VL	Ancient	TLN	Meso
44	264	com12	AM-A2	AM	Ancient	TLN	Meso
44	272	com5	AM-R2	AM	Post-agricultural	TLN	Eu
44	312	com7	OR-A2	OR	Ancient	TLN	Oligo
45	66	com4	SW-A2	SW	Ancient	T	Meso
45	178	com8	PR-A3	PR	Ancient	T	Oligo
45	242	com2	AM-A1	AM	Ancient	T	Meso
45	250	com7	AM-R1	AM	Post-agricultural	T	Eu
46	112	com10	EST-R1	EST	Post-agricultural	TLN	Meso
46	136	com7	EST-A3	EST	Ancient	TLN	Oligo
46	328	com9	OR-A3	OR	Ancient	TLN	Meso
46	360	com7	CH-A2	CH	Ancient	TLN	Eu
47	71	com10	SW-A2	SW	Ancient	LN	Meso
47	119	com4	EST-A2	EST	Ancient	LN	Oligo
47	207	com9	BR-R1	BR	Post-agricultural	LN	Oligo
47	327	com4	OR-A3	OR	Ancient	LN	Meso
48	45	com9	VL-R3	VL	Post-agricultural	TL	Meso
48	69	com5	SW-A2	SW	Ancient	TL	Meso
48	149	com12	PR-A1	PR	Ancient	TL	Oligo
48	341	com10	CH-A1	CH	Ancient	TL	Eu
49	22	com5	VL-A2	VL	Ancient	TN	Meso
49	70	com9	SW-A2	SW	Ancient	TN	Meso
49	142	com4	EST-R3	EST	Post-agricultural	TN	Oligo

Plot	Mesocosm	Community	Sample	Region	Land-use history	Treatment	Soil type
49	158	com3	PR-R1	PR	Post-agricultural	TN	Oligo
50	1	com3	VL-A1	VL	Ancient	C	Meso
50	81	com2	SW-A3	SW	Ancient	C	Oligo
50	265	com9	AM-R2	AM	Post-agricultural	C	Eu
50	337	com6	CH-A1	CH	Ancient	C	Eu
51	28	com8	VL-R2	VL	Post-agricultural	N	Meso
51	180	com10	PR-A3	PR	Ancient	N	Oligo
51	236	com12	BR-R3	BR	Post-agricultural	N	Oligo
51	356	com11	CH-A2	CH	Ancient	N	Eu
52	56	com1	SW-A1	SW	Ancient	TLN	Oligo
52	80	com12	SW-R2	SW	Post-agricultural	TLN	Meso
52	88	com10	SW-A3	SW	Ancient	TLN	Oligo
52	320	com2	OR-R2	OR	Post-agricultural	TLN	Oligo
53	39	com2	VL-A3	VL	Ancient	LN	Meso
53	47	com2	VL-R3	VL	Post-agricultural	LN	Meso
53	151	com8	PR-A1	PR	Ancient	LN	Oligo
53	303	com4	OR-R1	OR	Post-agricultural	LN	Oligo
54	86	com7	SW-A3	SW	Ancient	TN	Oligo
54	214	com12	BR-A2	BR	Ancient	TN	Oligo
54	294	com8	OR-A1	OR	Ancient	TN	Oligo
54	366	com2	CH-R2	CH	Post-agricultural	TN	Eu
55	36	com5	VL-A3	VL	Ancient	N	Meso
55	68	com2	SW-A2	SW	Ancient	N	Meso
55	156	com5	PR-R1	PR	Post-agricultural	N	Oligo
55	244	com6	AM-A1	AM	Ancient	N	Meso
56	105	com9	EST-R1	EST	Post-agricultural	C	Meso
56	169	com7	PR-R2	PR	Post-agricultural	C	Oligo
56	177	com12	PR-A3	PR	Ancient	C	Oligo
56	233	com3	BR-R3	BR	Post-agricultural	C	Oligo
57	194	com11	BR-A1	BR	Ancient	T	Oligo
57	226	com12	BR-A3	BR	Ancient	T	Oligo
57	330	com11	OR-R3	OR	Post-agricultural	T	Meso
57	362	com4	CH-R2	CH	Post-agricultural	T	Eu
58	91	com12	SW-R3	SW	Post-agricultural	L	Meso
58	99	com3	EST-A1	EST	Ancient	L	Meso
58	107	com1	EST-R1	EST	Post-agricultural	L	Meso
58	307	com8	OR-A2	OR	Ancient	L	Oligo
59	16	com5	VL-R1	VL	Post-agricultural	TLN	Meso
59	120	com8	EST-A2	EST	Ancient	TLN	Oligo
59	224	com9	BR-R2	BR	Post-agricultural	TLN	Oligo
59	304	com6	OR-R1	OR	Post-agricultural	TLN	Oligo
60	135	com10	EST-A3	EST	Ancient	LN	Oligo
60	167	com5	PR-A2	PR	Ancient	LN	Oligo
60	287	com4	AM-R3	AM	Post-agricultural	LN	Meso
60	383	com6	CH-R3	CH	Post-agricultural	LN	Eu
61	176	com3	PR-R2	PR	Post-agricultural	TLN	Oligo
61	184	com11	PR-A3	PR	Ancient	TLN	Oligo
61	192	com6	PR-R3	PR	Post-agricultural	TLN	Oligo
61	232	com5	BR-A3	BR	Ancient	TLN	Oligo
62	2	com1	VL-A1	VL	Ancient	T	Meso
62	34	com1	VL-A3	VL	Ancient	T	Meso
62	90	com3	SW-R3	SW	Post-agricultural	T	Meso
62	146	com3	PR-A1	PR	Ancient	T	Oligo
63	94	com6	SW-R3	SW	Post-agricultural	TN	Meso
63	326	com2	OR-A3	OR	Ancient	TN	Meso
63	334	com2	OR-R3	OR	Post-agricultural	TN	Meso
63	350	com12	CH-R1	CH	Post-agricultural	TN	Eu
64	41	com10	VL-R3	VL	Post-agricultural	C	Meso
64	73	com1	SW-R2	SW	Post-agricultural	C	Meso
64	209	com8	BR-A2	BR	Ancient	C	Oligo
64	345	com2	CH-R1	CH	Post-agricultural	C	Eu
65	13	com2	VL-R1	VL	Post-agricultural	TL	Meso
65	29	com2	VL-R2	VL	Post-agricultural	TL	Meso
65	165	com3	PR-A2	PR	Ancient	TL	Oligo
65	237	com7	BR-R3	BR	Post-agricultural	TL	Oligo
66	93	com8	SW-R3	SW	Post-agricultural	TL	Meso
66	205	com1	BR-R1	BR	Post-agricultural	TL	Oligo
66	269	com4	AM-R2	AM	Post-agricultural	TL	Eu
66	381	com8	CH-R3	CH	Post-agricultural	TL	Eu

Plot	Mesocosm	Community	Sample	Region	Land-use history	Treatment	Soil type
67	51	com12	SW-A1	SW	Ancient	L	Oligo
67	115	com6	EST-A2	EST	Ancient	L	Oligo
67	139	com6	EST-R3	EST	Post-agricultural	L	Oligo
67	219	com5	BR-R2	BR	Post-agricultural	L	Oligo
68	23	com3	VL-A2	VL	Ancient	LN	Meso
68	159	com9	PR-R1	PR	Post-agricultural	LN	Oligo
68	295	com12	OR-A1	OR	Ancient	LN	Oligo
68	359	com1	CH-A2	CH	Ancient	LN	Eu
69	228	com1	BR-A3	BR	Ancient	N	Oligo
69	260	com2	AM-A2	AM	Ancient	N	Meso
69	292	com3	OR-A1	OR	Ancient	N	Oligo
69	300	com5	OR-R1	OR	Post-agricultural	N	Oligo
70	19	com7	VL-A2	VL	Ancient	L	Meso
70	27	com4	VL-R2	VL	Post-agricultural	L	Meso
70	83	com3	SW-A3	SW	Ancient	L	Oligo
70	331	com8	OR-R3	OR	Post-agricultural	L	Meso
71	126	com11	EST-R2	EST	Post-agricultural	TN	Oligo
71	262	com10	AM-A2	AM	Ancient	TN	Meso
71	286	com9	AM-R3	AM	Post-agricultural	TN	Meso
71	374	com8	CH-A3	CH	Ancient	TN	Eu
72	106	com11	EST-R1	EST	Post-agricultural	T	Meso
72	290	com7	OR-A1	OR	Ancient	T	Oligo
72	298	com8	OR-R1	OR	Post-agricultural	T	Oligo
72	322	com10	OR-A3	OR	Ancient	T	Meso
73	35	com3	VL-A3	VL	Ancient	L	Meso
73	235	com10	BR-R3	BR	Post-agricultural	L	Oligo
73	363	com10	CH-R2	CH	Post-agricultural	L	Eu
73	371	com1	CH-A3	CH	Ancient	L	Eu
74	32	com12	VL-R2	VL	Post-agricultural	TLN	Meso
74	48	com6	VL-R3	VL	Post-agricultural	TLN	Meso
74	96	com9	SW-R3	SW	Post-agricultural	TLN	Meso
74	256	com3	AM-R1	AM	Post-agricultural	TLN	Eu
75	5	com10	VL-A1	VL	Ancient	TL	Meso
75	117	com3	EST-A2	EST	Ancient	TL	Oligo
75	197	com12	BR-A1	BR	Ancient	TL	Oligo
75	357	com2	CH-A2	CH	Ancient	TL	Eu
76	74	com10	SW-R2	SW	Post-agricultural	T	Meso
76	170	com9	PR-R2	PR	Post-agricultural	T	Oligo
76	266	com6	AM-R2	AM	Post-agricultural	T	Eu
76	274	com7	AM-A3	AM	Ancient	T	Meso
77	17	com1	VL-A2	VL	Ancient	C	Meso
77	185	com4	PR-R3	PR	Post-agricultural	C	Oligo
77	201	com4	BR-R1	BR	Post-agricultural	C	Oligo
77	289	com4	OR-A1	OR	Ancient	C	Oligo
78	110	com3	EST-R1	EST	Post-agricultural	TN	Meso
78	174	com6	PR-R2	PR	Post-agricultural	TN	Oligo
78	230	com4	BR-A3	BR	Ancient	TN	Oligo
78	278	com6	AM-A3	AM	Ancient	TN	Meso
79	84	com6	SW-A3	SW	Ancient	N	Oligo
79	164	com12	PR-A2	PR	Ancient	N	Oligo
79	196	com7	BR-A1	BR	Ancient	N	Oligo
79	220	com6	BR-R2	BR	Post-agricultural	N	Oligo
80	168	com6	PR-A2	PR	Ancient	TLN	Oligo
80	240	com11	BR-R3	BR	Post-agricultural	TLN	Oligo
80	376	com2	CH-A3	CH	Ancient	TLN	Eu
80	384	com4	CH-R3	CH	Post-agricultural	TLN	Eu
81	12	com3	VL-R1	VL	Post-agricultural	N	Meso
81	100	com9	EST-A1	EST	Ancient	N	Meso
81	188	com8	PR-R3	PR	Post-agricultural	N	Oligo
81	348	com3	CH-R1	CH	Post-agricultural	N	Eu
82	75	com9	SW-R2	SW	Post-agricultural	L	Meso
82	259	com11	AM-A2	AM	Ancient	L	Meso
82	291	com1	OR-A1	OR	Ancient	L	Oligo
82	347	com7	CH-R1	CH	Post-agricultural	L	Eu
83	208	com8	BR-R1	BR	Post-agricultural	TLN	Oligo
83	296	com11	OR-A1	OR	Ancient	TLN	Oligo
83	344	com4	CH-A1	CH	Ancient	TLN	Eu
83	352	com1	CH-R1	CH	Post-agricultural	TLN	Eu
84	10	com12	VL-R1	VL	Post-agricultural	T	Meso

Plot	Mesocosm	Community	Sample	Region	Land-use history	Treatment	Soil type
84	162	com8	PR-A2	PR	Ancient	T	Oligo
84	202	com2	BR-R1	BR	Post-agricultural	T	Oligo
84	234	com6	BR-R3	BR	Post-agricultural	T	Oligo
85	101	com4	EST-A1	EST	Ancient	TL	Meso
85	125	com7	EST-R2	EST	Post-agricultural	TL	Oligo
85	245	com10	AM-A1	AM	Ancient	TL	Meso
85	301	com7	OR-R1	OR	Post-agricultural	TL	Oligo
86	121	com2	EST-R2	EST	Post-agricultural	C	Oligo
86	281	com6	AM-R3	AM	Post-agricultural	C	Meso
86	369	com11	CH-A3	CH	Ancient	C	Eu
86	377	com11	CH-R3	CH	Post-agricultural	C	Eu
87	102	com6	EST-A1	EST	Ancient	TN	Meso
87	246	com7	AM-A1	AM	Ancient	TN	Meso
87	254	com12	AM-R1	AM	Post-agricultural	TN	Eu
87	358	com3	CH-A2	CH	Ancient	TN	Eu
88	4	com2	VL-A1	VL	Ancient	N	Meso
88	44	com7	VL-R3	VL	Post-agricultural	N	Meso
88	124	com4	EST-R2	EST	Post-agricultural	N	Oligo
88	316	com8	OR-R2	OR	Post-agricultural	N	Oligo
89	60	com10	SW-R1	SW	Post-agricultural	N	Oligo
89	172	com11	PR-R2	PR	Post-agricultural	N	Oligo
89	324	com1	OR-A3	OR	Ancient	N	Meso
89	364	com6	CH-R2	CH	Post-agricultural	N	Eu
90	87	com5	SW-A3	SW	Ancient	LN	Oligo
90	231	com6	BR-A3	BR	Ancient	LN	Oligo
90	239	com8	BR-R3	BR	Post-agricultural	LN	Oligo
90	375	com3	CH-A3	CH	Ancient	LN	Eu
91	25	com11	VL-R2	VL	Post-agricultural	C	Meso
91	89	com4	SW-R3	SW	Post-agricultural	C	Meso
91	297	com9	OR-R1	OR	Post-agricultural	C	Oligo
91	353	com5	CH-A2	CH	Ancient	C	Eu
92	63	com3	SW-R1	SW	Post-agricultural	LN	Oligo
92	183	com9	PR-A3	PR	Ancient	LN	Oligo
92	263	com3	AM-A2	AM	Ancient	LN	Meso
92	335	com10	OR-R3	OR	Post-agricultural	LN	Meso
93	61	com1	SW-R1	SW	Post-agricultural	TL	Oligo
93	221	com7	BR-R2	BR	Post-agricultural	TL	Oligo
93	349	com5	CH-R1	CH	Post-agricultural	TL	Eu
93	373	com12	CH-A3	CH	Ancient	TL	Eu
94	147	com11	PR-A1	PR	Ancient	L	Oligo
94	179	com7	PR-A3	PR	Ancient	L	Oligo
94	251	com6	AM-R1	AM	Post-agricultural	L	Eu
94	355	com6	CH-A2	CH	Ancient	L	Eu
95	127	com1	EST-R2	EST	Post-agricultural	LN	Oligo
95	247	com1	AM-A1	AM	Ancient	LN	Meso
95	319	com7	OR-R2	OR	Post-agricultural	LN	Oligo
95	351	com11	CH-R1	CH	Post-agricultural	LN	Eu
96	130	com6	EST-A3	EST	Ancient	T	Oligo
96	354	com10	CH-A2	CH	Ancient	T	Eu
96	370	com9	CH-A3	CH	Ancient	T	Eu
96	378	com9	CH-R3	CH	Post-agricultural	T	Eu

Appendix 3.4. Soil moisture content

This appendix describes the measurement of volumetric soil moisture content in all mesocosms, both in a dry period and after a rainfall event.

Methods

We measured the volumetric soil moisture content ($m^3\ m^{-3}$) in all mesocosms after a dry period in September 2016 (13/09, no rainfall for 7 days) and after a rainfall event on October 3rd 2016. We used a Delta T ML3 Thetakit (Delta T, Cambridge UK), with two measurements in each mesocosm (one centre, one edge). We analysed the data using a linear mixed effects model with the volumetric soil moisture content as response, with rainfall event (2 levels), Soiltype (3 levels), Warming (2 levels), light addition (2 levels), N addition (2 levels), Land-use history (2 levels) as fixed main effects and the Plot number as the only random effect.

Results

Table A3.4.1. Mean volumetric soil moisture content ($m^3\ m^{-3}$) in all mesocosms. These were analysed on main effects for rainfall event, soil type, land-use history. The intercept value is the soil moisture content on a dry day, on a “Eutrophic” soil with “Ancient” land-use history, and no warming, light or N addition applied.

term	estimate	standard error	t statistic	p value
(Intercept)	41.17	0.87	47.36	**p < 0.001**
Rainfall event	+1.64	0.33	4.99	**p< 0.001**
Soil type				
Mesotrophic	+1.81	0.54	3.32	**p < 0.001**
Oligotrophic	-3.68	0.53	-6.97	**p < 0.001**
Land-use history				
Post-agricultural	-3.33	0.36	-9.26	**p< 0.001**
Warming	-0.3	0.71	-0.43	0.67
Light	-0.79	0.71	-1.10	0.27
Nitrogen	-0.8	0.71	-1.13	0.26

Appendix 3.5. Mortality counts and plant survival

Methods

The score to assess the plant performance was based on the growth stage of the individual plants (Figure A3.5.1). This continuous aging-scale was discretely classified into 5 classes:

1. Vegetative growth. Bud burst, juvenile growth with green leaves but no reproductive parts.
2. Reproductive growth. Plant showing reproductive parts: flower buds, flowering, seeding. Leaves and other plant organs in maturation stage. Considered as the prime period for measuring plant functional traits as this will be the moment of peak biomass.
3. Senescence. Yellowing of leaves, loosening of seeds.
4. Death.
5. Non- emergent or missing (not visible on the scheme in Figure A3.5.1).

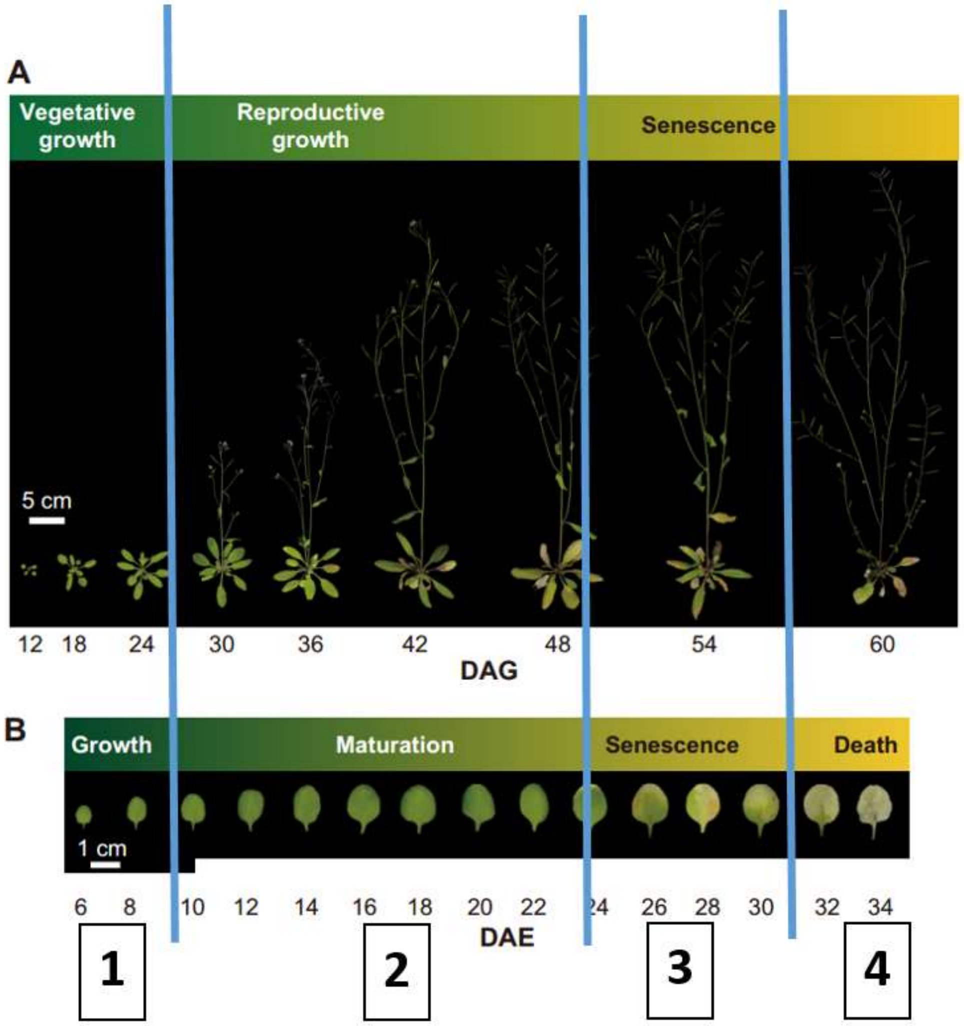


Figure A3.5.1. Link between growth stages, senescence of leaves and the ordinal scores. These scores are: 1 (Vegetative growth), 2 (Reproductive growth and maturation), 3 (signs of senescence in leaves) and 4 (death).The figure is adapted from Woo et al. (2013), showing Arabidopsis thaliana. DAG is the abbreviation for “days after germination”, DAE for “days after emergence”.

Results

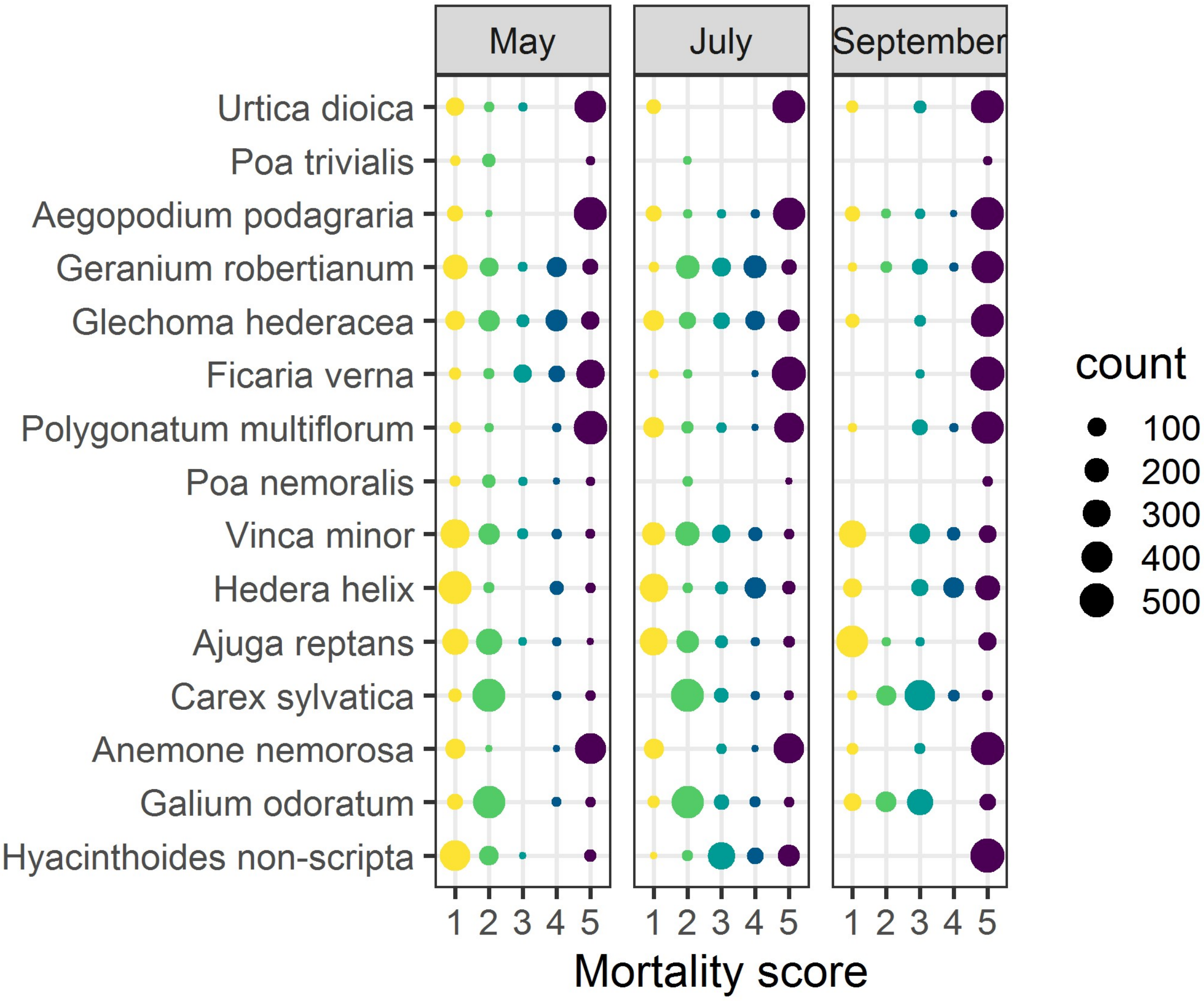


Figure A3.5.2. The mortality score (see Figure A3.5.1) for each species at three occasions in 2016 (early May, early July and early September). Species are ordered on CCI, with fast colonisers on top. Each species has a total count of 512 individuals. The performance counts in May 2016 occurred prior to replanting. Category 1 (vegetative) and category 2 (regenerative phase) are considered healthy. We replanted individuals in category 3 (senescence), 4 (dead) and 5 (missing) in the subsequent week. Notice that *Poa nemoralis* and *Poa trivialis* show irregular patterns in mortality scores. The coexisting *Poa nemoralis* and *Poa trivialis* in two species combinations (64 mesocosms, see Table A3.1.1) precluded identification of the young and emerging graminoids, so that grass cover of both species were considered equal when coexisting. We resolved this by replanting *Poa* individuals during the weeding process in September 2016, when grasses had developed identifying characteristics i.e. flowers.


Image: Sofie Verstraelen

Chapter 4

Individualistic responses of forest herb traits to environmental change

Adapted from: Haben Blondeel, Michael P. Perring, Emiel De Lombaerde, Leen Depauw, Dries Landuyt, Sanne Govaert, Sybryn L. Maes, Pieter Vangansbeke, Pieter De Frenne, Kris Verheyen. *Individualistic responses of forest herb traits to environmental change.* Resubmitted to Plant Biology.

Abstract

Intraspecific trait variation (ITV; i.e. variability in the mean and/or distribution of plant attrivute values within species), can occur in response to multiple drivers. Environmental change and land-use legacies could alter trait values within species directly, but also indirectly by changing the vegetation cover. Greater variability in environmental conditions could lead to more ITV but responses could differ among species. Disentangling these drivers on ITV is necessary to accurately predict plant community responses to global change. We planted herb communities into forest soils with and without recent agricultural history. Soils were collected from across temperate Europe, while the fifteen selected herb species had different colonising abilities and affinities to forest habitat. These mesocosms (384) were exposed to two-level full-factorial treatments of warming, nitrogen addition and illumination. We measured plant height and specific leaf area (SLA). For the majority of species, mean plant height values increased as vegetation cover increased in response to light addition, warming and agricultural legacies. The coefficient of variation (CV) for plant height was larger in fast colonising species. Mean SLA for vernal species increased with warming, while light addition mainly decreased mean SLA for shade-tolerant species. Interactions between treatments were unimportant predictors. Environmental change treatments influenced ITV, either via an increasing vegetation cover or by affecting trait values directly. Species' ITV was individualistic i.e. species responded to different single resource and condition manipulations that benefited their growth in the short term. These individualistic responses could be important for altered community organisation after a prolonged period.

Introduction

Functional traits are morphological, physiological or phenological traits which affect growth, reproduction, and survival of plants (Funk et al., 2017; Violle et al., 2007). Variation in functional traits related to leaf economics ("leaf"), growth ("height") and reproduction ("seed") has been associated with trade-offs that plant species face to compete for resources across environmental gradients (Beckman et al., 2018; Borer et al., 2014; Díaz et al., 2016; Reich, 2014; Westoby, 1998). While many have focused on trait variation between species and how different environments influence this (Bruelheide et al., 2018; Wright et al., 2017), trait variation also occurs within species (Fajardo & Siefert, 2018; Siefert et al., 2015). This intraspecific trait variation (ITV) can occur in response to environmental drivers (Albert et al., 2010; Jung, Violle, Mondy, Hoffmann, & Muller, 2010), and is maintained by processes of phenotypic plasticity and genetic differentiation (Valladares et al., 2006). Phenotypic plasticity is an individual's plastic response to its environment, and its effect can be larger than that of genetic adaptation in altering trait values of species at small spatiotemporal scales (Lajoie & Vellend, 2015, 2018; Turcotte & Levine, 2016). Changes in mean trait values of species in response to altered environments can ultimately affect variability in traits across populations, and thus contribute to ITV (Moran et al., 2016). ITV greatly influences community patterns and processes (Bolnick et al., 2011; Escudero & Valladares, 2016), and accounting for these effects improves predictions of plant communities in a changing world (Cadotte, Arnillas, Livingstone, & Yasui, 2015; Laughlin, Joshi, van Bodegom, Bastow, & Fulé, 2012).

Evidence exists that competition for light enhances ITV in height and leaf trait values (Bennett, Riibak, Tamme, Lewis, & Pärtel, 2016; Henneron et al., 2017; Le Bagousse-Pinguet et al., 2015). This is most evident in asymmetric competition for light, where light as a resource is disproportionally removed by individuals that manage to grow taller than their direct neighbours (Freckleton & Watkinson, 2001). In dense plant communities with high cover, larger growth of individuals can be achieved as long as additional mineral nutrition and water are provided to sustain the increased growth (Begon, Harper, & Townsend, 1996; Craine & Dybzinski, 2013; Freckleton & Watkinson, 2001; Watkinson, 1983). Asymmetric competition for light in such dense communities shapes the structure of canopies (Nagashima & Hikosaka, 2011) and the plant community as a whole (DeMalach et al., 2016). Plants that manage to survive under the shade of the taller neighbours can alter leaf morphology to deal with photosynthesis in low light environments (Valladares & Niinemets, 2008). A common plastic response of individual plants is to increase the leaf area to leaf mass ratio (specific leaf area, SLA) to optimize light capture for growth maintenance (Liu et al., 2016; Milla & Reich, 2007; Smart et al., 2017; Valladares & Niinemets, 2008). Plants can also compete for light that is inherently scarce, such as in herbaceous communities at the forest floor under the shade of large canopy trees (Valladares et al., 2016). Sudden gaps in the tree canopy could then enhance light availability and photosynthesis in the understorey at very short notice (Paul-Limoges, Wolf, Eugster, Hörtnagl, & Buchmann, 2017). This change in light environment could lead to quick plastic responses in plant height and SLA (Valladares, Balaguer, Martinez-Ferri, Perez-Corona, & Manrique, 2002) in synchrony with shifts in community composition (De Frenne et al., 2015) and an increased community biomass production (Jagodziński et al., 2016).

Apart from a variable light environment (Burton, Perakis, McKenzie, Lawrence, & Puettmann, 2017; Garnier, Navas, et al., 2016b; Lemke et al., 2015; Navas & Garnier, 2002; Rozendaal et al., 2006), three main global change drivers are affecting temperate forest herb ecology (Gilliam, 2007) and thus potentially ITV. First, climate warming can alter growth and leaf trait values within herbaceous plants to resemble those of warmth-adapted communities (Bjorkman et al., 2018; De Frenne et al., 2015; Helsen et al., 2017; Henn et al., 2018; Hoeppner & Dukes, 2012). Second, nitrogen (N) enrichment from atmospheric depositions (Bobbink et al., 2010) can cause increasing mean values of plant height and SLA by enhancing growth and increasing N content per dry mass of plant tissues (Hejcman, Křišťálová, Červená, Hrdličková, & Pavlů, 2012; Mao et al., 2017). Finally, enrichment in soil N and phosphorus (P) stemming from agricultural legacies can cause increases in productivity and variability in functional leaf traits (Baeten, Vanhellemont, De Frenne, De Schrijver, et al., 2010; Baeten et al., 2011; Fraterrigo et al., 2009; Siefert & Ritchie, 2016). These drivers can interact to affect trait values in plant communities (Perring et al., 2016). Furthermore, plant responses to multiple environmental changes can differ between species and the trait of interest, controlled by a species' ecological strategy (Garnier, Navas, et al., 2016b; Jagodziński et al., 2016; Mao et al., 2017; Poorter et al., 2009).

Plant functional traits can differ between species depending on how species capture resources (Garnier, Navas, et al., 2016b; Jagodziński et al., 2016; Mao et al., 2017; Poorter et al., 2009). In forest herbs, there is evidence that life-history traits and plant height are correlated to forest colonisation and specialisation in forests habitat use (Verheyen, Honnay, et al., 2003). Herbaceous species that are confined to ancient forests are typically small-statured slow colonisers that can photosynthesise in mature forests, either as spring-flowering geophytes (Mabry et al., 2008; Rothstein, 2000; Tessier & Raynal, 2003) or as shade tolerant plants that grow under fully-developed tree canopies (Valladares & Niinemets, 2008). These perennial ancient forest herbs consequently invest more nutrients into roots, bulbs, suckers or large seeds for proliferation (Verheyen, Honnay, et al., 2003), but at a cost of slow dispersal (Klimešová et al., 2016). The ability of forest herbs to colonise post-agricultural forests correlates positively with large relative growth rates and aging quickly (Verheyen, Honnay, et al., 2003), both plant characteristics indicative of a "fast life-history" (Adler et al., 2014; Beckman et al., 2018). Such fast colonisers are tall with light seed which allows further dispersal than species that are typical of ancient forest (Beckman et al., 2018; Thomson, Moles, Auld, & Kingsford, 2011; Verheyen, Honnay, et al., 2003). Fast colonising forest herbs additionally have a high SLA and high leaf N content (Verheyen, Honnay, et al., 2003) which is a leaf construction that favours resource acquisition over resource conservation (Adler et al., 2014; Díaz et al., 2016; Reich, 2014; Wright et al., 2004). Fast colonisers could thus benefit more from variation in height and leaf traits than slow colonisers to quickly capitalise on resources in rapidly changing environments (Alpert & Simms, 2002).

Here we assess within-species responses of plant height and SLA to environmental change and land-use legacies directly, but also indirectly by looking at change in vegetation cover. Furthermore, responses could also differ between species and potentially link to colonisation capacity. We performed a manipulative experiment with temperate herbaceous communities in forests comprising species with varying colonisation capacities and affinities to forests habitat. This experiment allows us to disentangle effects of enhanced light availability, warming, N enrichment and agricultural legacies on intraspecific variation in plant height and SLA. We specifically hypothesize that:

1. Within species, high vegetation cover increases mean plant height and SLA, likely due to asymmetric competition for light. Higher vegetation cover could stem from community growth due to nutrient enrichment (from either N addition or agricultural legacies) in warmer and brighter conditions.
2. Within-species differences in mean plant height and SLA could result from variability in growing conditions induced by environmental changes and land-use legacies. The effect of these drivers can depend on each other. Concretely, we examine two-way interactions between enhanced light availability, warming, N enrichment and agricultural legacies on changes of mean values in plant height and SLA.
3. Species with a good ability to colonise post-agricultural forests can exhibit a greater variability in trait values for both plant height and SLA. Since these species colonise novel environments, they need to cope with higher environmental unpredictability, and could potentially benefit from larger trait variation than species confined to ancient forests.

Material and Methods

We performed a full-factorial experiment with 384 experimental units (mesocosms) to disentangle interactive two-level effects of forest land-use history, enhanced light availability, nitrogen addition and warming on fifteen common European herbaceous forest species that differ in colonisation capacity (Chapter 3). We measured plant height on 3445 individual plants and characterised SLA using 1125 leaf samples with measurements taken during the growing season of 2017, from the first week of March until the first week of September.

Experimental methods

We collected soil (0.1 m^3 in each forest) from eight temperate European regions across gradients of inherent soil fertility, regional phosphorus balance and nitrogen deposition (further details in Blondeel et al. 2019 and Chapter 2). Within each region, we searched for three adjacent pairs of ancient forest (in existence before 1850) and post-agricultural forest (established around 1950) with similar canopy composition, leading to 48 forest patches included in the study (Blondeel, Perring, et al., 2019). The agricultural legacies in the post-agricultural forest soils varied along gradients of inherent soil characteristics, regional P balance and N deposition (Blondeel, Perring, et al., 2019). Overall, the soils from post-agricultural forest had 0.4 units higher pH (+0.41 ± 0.16 standard error (S.E.)) than in ancient forest (mean ancient pH = 4.82 ± 0.33 S.E.). Soil C:N was lower (-1.49 ± 0.24 S.E.) in post agricultural forest than in ancient forest (mean ancient C:N = 13.9 ± 0.94 S.E.). Finally, Soil P concentrations were elevetad in post agricultural forest for total P (+118 mg kg^{-1} ± 60 S.E.) and Olsen P (+13 mg kg^{-1} ± 5.5 S.E.) compared to ancient forest (mean ancient total P = 347 mg kg^{-1} ± 64.5 S.E.; mean ancient Olsen P = 15.3 mg kg^{-1} ± 4.5 S.E.). The collected soils were classified in three groups using cluster analysis according to inherent soil fertility (texture and calcareous properties). These soil types were either i) "Eutrophic" which are rich in clay and carbonates with high pH (6.6-7.1), ii) "Oligotrophic" which are high in sand and low in pH (4 – 5) or iii) "Mesotrophic" with intermediate fertility, siltier textures and intermediate pH (5-5.6) (see Blondeel et al*.,* 2019). These soil groups are relative terms to categorise inherent soil fertility within our samples, and should not be used to compare outside of our population (further details in Blondeel et al. 2019 and Chapter 2). We use this categorical soil type variable as a covariate when testing variation in mean plant height and mean SLA in response to interactive environmental changes (see section "Data analysis").

These soils were used in a large mesocosm experiment, performed in a fenced area of the Aelmoeseneie forest (Gontrode, Belgium) since April 2016 (further details in Chapter 3). The mesocosms consist of five species with differing colonisation capacities and forest habitat use. We compiled a pool of fifteen species commonly found in temperate European forests (Chapter 3) and divided this species pool according to three emergent groups that are linked with a species' colonisation capacity (De Frenne, Baeten, et al., 2011; Verheyen, Honnay, et al., 2003) and whether a species can be considered as a forest specialist. The species in this pool can be characterised by the colonisation capacity index (CCI), where more negative values indicate a higher capacity to successfully colonise into post-agricultural forests (Verheyen, Honnay, et al., 2003). The continuous CCI scale exists from -100 to +100, where -100 means that the species only occurred in post-agricultural forest, while +100 means that the species was only found in ancient forest. At the time of planting, each mesocosm consisted of two poor colonisers and forest specialists, two intermediate colonisers of post-agricultural forest and not strictly forest specialists, and one fast-colonising nitrophilic species (see Chapter 3 for details on community assembly). These combinations of five species originated from twelve fixed species combinations that were randomly assembled (Chapter 3). These twelve species combinations were repeated four times over the 48 soil samples in each two level factorial combination of light x warming x nitrogen addition (48 soil samples x 8 factorial combinations = 384 mesocosms). In total, we planted 7680 (384 mesocosms x 20) individuals of fifteen species (512 individuals per species).

We have applied three two-level environmental treatments in a full-factorial design since April 2016 (Chapter 3). Ongoing treatments consist of (i) nitrogen enrichment; (ii) experimental warming and (iii) enhanced light availability. The first treatment is N enrichment with an additional 50 kg N ha^{-1} yr^{-1} eq., further referred to as treatment “N”. We applied the N treatment as four seasonal pulses per year, each time adding 0.25 L of a 2.01 g L-1 solution of NH_4NO_3 to each mesocosm and rinsing the leaves with 0.25 L of demineralised water. The control mesocosms received 0.5 L of demineralised water during each of the seasonal additions. The second treatment consisted of experimental warming (referred to as treatment “T”) using 75 cm-wide open top chambers (De Frenne et al., 2010). This treatment significantly raised the air temperate between 1°C and 2 ° C in spring 2017, but with insignificant temperature effects when the forest canopy closed towards the end of May (Chapter 3). The third treatment, light addition (referred to as “L”) adds around 25 µmol $m^{-2}s^{-1}$ PAR to the ambient light conditions (below 10 µmol $m^{-2}s^{-1}$ under fully closed canopy) by use of two 18 W fluorescent tubes suspended 75 cm above ground level of each plot (Chapter 3). These lights are programmed to follow the natural photoperiod throughout the year and did not significantly affect air temperatures (De Frenne et al., 2015).

Summarising the design of the 384 mesocosms, we have eight regional origins of soil (classified in three groups according to inherent soil fertility), with three ancient and three post-agricultural forest soils (LU) in each region (48 soil samples), using two-level treatments of N enrichment (N), warming (T) and light (L) addition (8 combinations).

Plant trait and community measurements

We measured plant height and SLA in the second growing season of the experiment, from March 2017 to September 2017, at the time of each species’ estimated biomass peak (see Table

3.1 in Chapter 3 for species' peak biomass). We chose not to measure reproductive traits (i.e. seed mass) as proposed in the common leaf-height-seed ecological strategy scheme (Laughlin et al., 2010; Westoby, 1998). Seed mass and plant height are strongly positively correlated in the global plant trait spectrum (Beckman et al., 2018; Díaz et al., 2016; Thomson, Moles, Auld, & Kingsford, 2011), but also in forest herb species (Verheyen, Honnay, et al., 2003). Reproductive traits within species can have ambiguous responses to multiple environmental drivers (Garnier, Navas, et al., 2016b), especially in herbaceous plants that rely more on below-ground organs for reproduction (Klimešová et al., 2016) such as common understorey herbs (Verheyen, Honnay, et al., 2003). Furthermore, these seed traits are labour intensive to correctly measure and quantify given our large numbers of test plants (Pérez-Harguindeguy et al., 2013).

We were able to measure plant height on the 3445 survivors of the 7680 initially planted individuals (45% survival, Table A4.1.1). The number of plant height measurements (i.e. individual survivors) did not depend on treatment effects (Figure A4.1.1). Differences in survival among species existed (Table A4.1.1). Vegetative plant height was measured as the shortest distance between ground level and the upper boundary of main photosynthetic tissues. This means that we solely measured foliage height, not inflorescence or seeding height (Pérez-Harguindeguy et al., 2013). We measured plant height using a common folding meter in centimetres up to one decimal place.

We measured average specific leaf area (SLA) as the one-sided area of multiple leaves from a single species in each mesocosm, divided by the oven-dry mass and expressed in $mm^2\ mg^{-1}$ (Pérez-Harguindeguy et al., 2013). We collected maximally eight leaves of each species and no more than two per individual to avoid excessive damage to any individual plant. We collected fully expanded and hardened leaves from adult plants (which emerged in growing season of 2017), cutting each leaf from the stem but not including a petiole where present. We avoided leaves with pathogen or herbivore damage. Because SLA is strongly affected by light intensity, we only sampled outer leaves (Pérez-Harguindeguy et al., 2013). The leaves were collected in flasks with a few drops of deionized water under high CO_2 conditions (breathing in flask before sealing) to maintain turgor and prevent wilting. Prior to measuring leaf area, we gently patted dry each leaf. The amount of SLA measurements did not vary due to treatments within any species (Figure A4.1.2), while there were larger differences in measured SLA values between species (Table A4.1.1). The projected area of the leaves was measured with the *Easy leaf area free* mobile application (Easlon & Bloom, 2014) within two hours of picking the leaves, after taking a picture with a mobile phone (13 MP camera). This open-source software package can estimate green leaf area in a RGB photograph by counting the total amount of green pixels and converting this into an area measurement by counting red pixels from a 4 cm^2 calibration square provided in the image. After the area measurement (mm²), each leaf sample was dried in an oven at 65°C for 48 hours to ensure constant mass upon weighing (in mg).

We measured total vegetation cover (%), which is tightly linked with productivity, leaf biomass and competition for light (Muukkonen et al., 2006). We measured vegetation cover as the one-sided projection of vegetation in the tray. Logically, 0% means that there is no vegetation and 100% cover means that the whole tray area was covered by vegetation. We measured total cover two times during the experiment: the first week of May (4th of May) and the second week of August (11th August). We used digital RGB photographs of the mesocosms taken perpendicular to the ground surface and the *Canopy Area* tool that measures green pixels of vegetation and recalculates this into a cover percentage (Easlon & Bloom, 2014). Consequently, species abundances are not taken into account in this Chapter, as we focus on individualistic responses of the understorey plants.

Data analysis

All data analysis was performed in R (R Core Team, 2019). We performed three analyses to assess whether trait variation in plant height and SLA within species is a function of 1) vegetation cover, 2) land-use legacies interacting with multiple environmental drivers and 3) a species' colonisation ability. We consequently addressed within-species trait variation in two ways. First, by assessing change in mean trait values due to vegetation cover and treatments (hypothesis 1 and 2 respectively). Second, by looking at variability in traits across all measured values via the coefficient of variation (CV, hypothesis 3). Prior to the analyses, we excluded *Glechoma hederacea* because this species had fewer than 30 measurements (see Chapter 3, Table A4.1.1). During the analysis on variation in mean plant height and SLA (hypothesis 1 and 2), we excluded *Geranium robertianum* because there were too few measurements to fit the model structure robustly.

In the first analysis, we tested whether vegetation cover explains differences in mean plant height and SLA in each species (hypothesis 1). We calculated separate hierarchical linear mixed – effects models for each species to estimate the mean trait value in response to vegetation cover. The original Region of the soil (8 levels), Community (12 levels) and Plot (96 levels) are separate random effect terms in this structure, i.e. (1|Region) + (1|Community) + (1|Plot). Adding the "Plot" random effect term was not possible for SLA because of limited degrees of freedom (SLA is an averaged measure for each species in each mesocosm). The predictor variable is the average vegetation cover (%) of each mesocosm measured between May 2017 and August 2017. This averaged measure allows to characterise the vegetation cover of a community with a single metric that is relevant for all species during the whole growing season. We checked normality in residuals with a Shapiro-Wilk test on each model, and log-transformed the response variable when necessary to achieve normality in model residuals. Log transforming the response was needed for six species in plant height and five species in SLA (see Table A4.1.2). We additionally tested whether the covariate (Soil type), and two-way interactions (including main effects) of land-use history (LU), enhanced light availability (L), nitrogen addition (N) and warming (T) directly explained variation in total vegetation cover. We used the same hierarchical model structure as explained above.

We tested the second hypothesis by estimating mean plant height and SLA of species in response to two-way interactions of land-use history (LU), enhanced light availability (L), nitrogen addition (N) and warming (T). Concretely, we calculated separate hierarchical linear mixed effects models for each species with six double interactions including all four main effects (LU, L, N, T, LU:L, LU:N, LU:T, L:N, L:T, N:T) and an extra covariate "Soil type". We included the covariate "Soil type" (three levels: "eutrophic", "mesotrophic", "oligotrophic") in the models because inherent soil fertility can be a major source of trait variation, even though it is not one of our focal environmental change predictors. The random effects structure of these models is the same as in hypothesis 1, with "Region" (8 levels), "Community combination" (12 levels) and "Plot" (96 levels) included. Prior to computing the tests, we checked normality assumptions using a Shapiro-Wilk test on each model accompanied by a qq-plot and histogram on model residuals. We log-transformed when necessary to achieve normality in residuals, which was the case for seven species for plant height and six species for SLA (see Table A4.1.3). We also checked the model plots of residuals vs fitted values to check for inconsistencies given the factors included in the model, which we did not observe. We did not check the models for independence of residuals to treatment effects, due to a too large amount of figures to check (2 traits x 13 species x 11 terms = 286 boxplot figures). We rather adopted a lower p-value threshold ($p < 0.007$) in the analysis to reduce the sensitivity of the results to outliers. We performed ANOVA (F-test) on one linear model for each of the thirteen species per trait, which contain 11 terms per model (four main effects, six double interactions and one covariate main effect). We applied an adjusted alpha ($p < 0.007$) so that the expected value of a false positive effect is less than 1 out of the 143 tested terms ($\alpha = 1/143 = 0.007$) for a given trait. If we had used the common alpha of $p < 0.05$, the expected value of interpreting false positive effects would have been 7 terms ($143*0.05 = 7.15$) for each trait.

We tested the third hypothesis by calculating the coefficient of variation (CV) for each species across all measurements of plant height and SLA. We performed linear regression of the CV as function of the species' capacity to colonise post-agricultural forest. We used the colonisation capacity index (CCI) to determine a species ability to successfully colonise post-agricultural forest (Verheyen, Honnay, et al., 2003), where more negative values denote a larger capacity to colonise post-agricultural forest. We calculated the coefficient of variation (CV) in plant height and SLA for the whole range of species' trait values (so across all treatments) as the ratio of the standard deviation to the estimate of the population mean. The CV is a simple measure for intraspecific trait variation and phenotypic plasticity in the wide sense, i.e. when traits are not measured on individuals of the same genotype but of the same species (Valladares et al., 2006). As we have computed one CV for each species for the whole data set, we performed a non-hierarchical linear regression of the species' coefficient of variation in response to the species' CCI for both traits (alpha =0.05).

Results

Response of mean traits to vegetation cover

In the first analysis, we found that increasing total vegetation cover (vegetation density) related to changes in mean values of both plant height and SLA. Vegetation cover (Table 4.1) was significantly higher on post-agricultural forest soils (+6.06 % cover, S.E. = 1.68), with light addition using fluorescent tubes (+15.88% cover, S.E. = 1.79) and with warming using open top chambers (+8.41 % cover, S.E. = 1.83). The average value in ancient forest soils with no treatment was 43.15 ± 3.4 % cover. There was no evidence for interactions between environmental treatments in affecting vegetation cover.

Table 4.1. Model fit of the significant ($p<0.05$) treatments that influenced mean vegetation cover (%). Estimates with standard error (S.E.), denominator degrees of freedom (D.F.), F-value and p-value are given for the model terms. The three fixed effect terms were the two-level terms Land-use history (LU), Light treatment (L) and Temperature treatment (T) which together explained less than 20% of variation ($R^2m = 0.17$). 65% of the variation is accounted for when including the random effect terms (R^2c), i.e. Region of soil, Plot and community combination. The intercept term is the average intercept across random effect terms and is the estimate for an Ancient forest soil with control treatments for light and warming.

Term	Estimate	S.E.	D.F.	F-value	p-value
Intercept	43.15	3.40	276	376	<0.0001
Post-agricultural	+6.06	1.68	9	12	0.0074
Light addition	+15.88	1.79	276	79	<0.0001
Warming	+8.41	1.83	276	21	<0.0001

In turn, we found that vegetation cover affected both species' mean plant height and mean SLA while mean plant height was more responsive to total vegetation cover than mean SLA (Figure 4.1). Ten of the thirteen species had increasing mean plant height estimates with increasing vegetation cover, all on $p < 0.001$. Estimates of the slopes are given in Table A4.1.2.

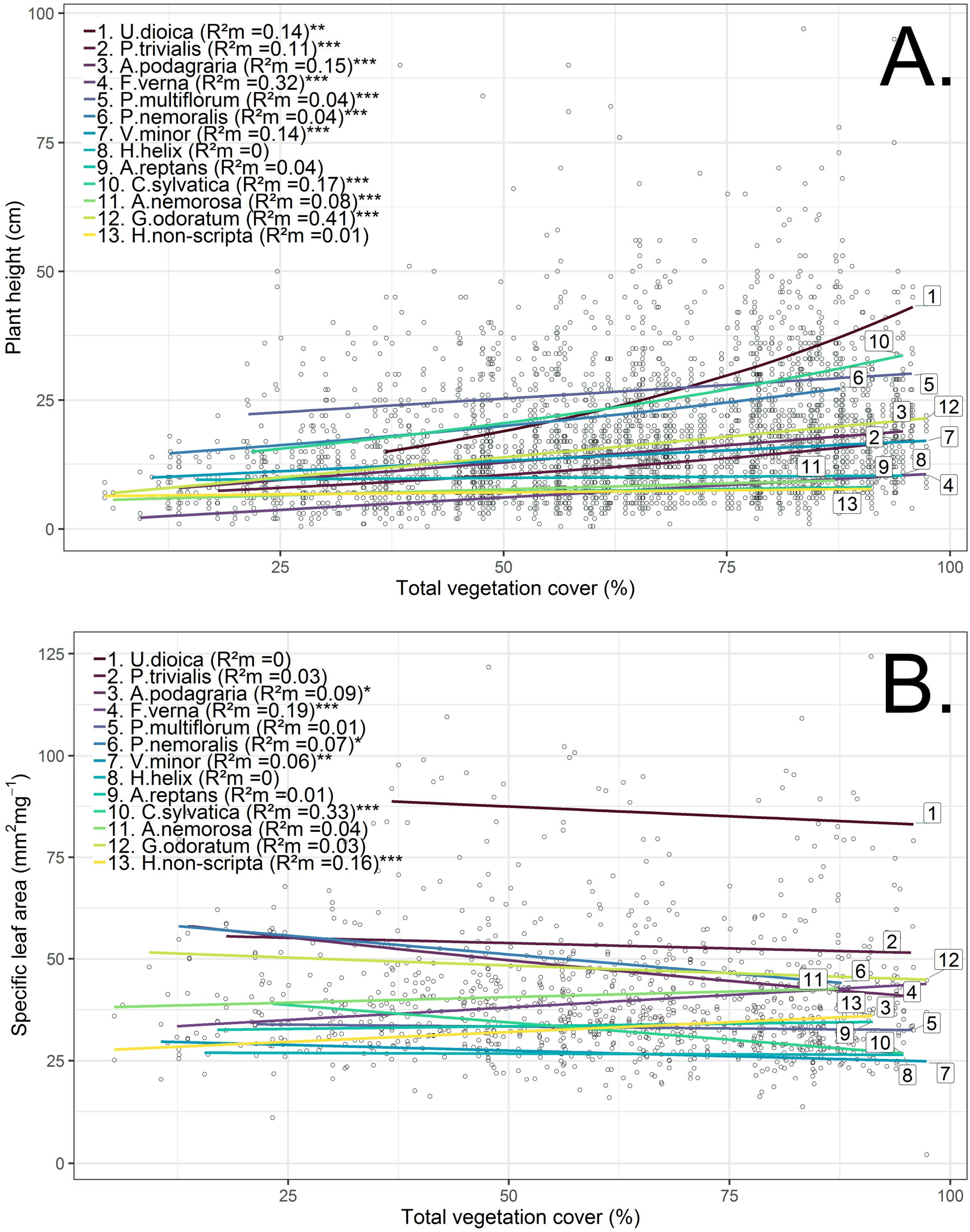


Figure 4.1. Plant height (panel A) is more responsive to total vegetation cover than SLA (panel B) across species. Results of the within species linear mixed effects models testing plant height and SLA in response to total vegetation cover in a mesocosm. Significance levels are 0.05*, 0.01** and 0.001***. The lines are the average predicted values of the linear mixed effects model for each species (either normal or log-transformed response). Marginal R^2 (R^2m) are given, which denote the model fit on the only fixed effect (vegetation cover). Species are ordered on colonisation capacity index (CCI) with the fastest colonisers into post-agricultural forest on top and hues ranging from purple-blue-yellow from fast to slow colonisers.

The largest absolute increase in plant height occurred in *Urtica dioica,* also the fastest coloniser into post-agricultural forest (most negative CCI). Its predicted mean plant height at the lowest community cover (36%) was 15.8 cm (95% confidence interval (CI): 8.4– 29.7 cm) and 41.6 cm (95% CI: 22.2 - 78.0 cm) at the highest community cover (96%). This is, over the range of total cover values that it occurred in, a relative increase in mean plant height of 160%. However, the largest relative increase was a five-fold increase in mean plant height that occurred in *Ficaria verna*. The model results show that this species increased its mean plant height from 1.9 cm (95% CI: 1.05 – 2.83 cm) at 9 % total cover to 10.6 cm (95% CI: 8.7 – 12.5 cm) when the total vegetation cover was 97 %.

For SLA, we found both significant increases and decreases in response to community vegetation cover depending on the species (Figure 4.1B). Species mean SLA decreased significantly in four species (*Aegopodium podagraria, Poa nemoralis, Vinca minor, Carex sylvatica*) and increased in two (*Ficaria verna, Hyacinthoides non-scripta*). However, the slopes were not as pronounced as for the plant height response (Figure 2) with 30 % as a maximal decrease of SLA for both *Aegopodium podagraria* and *Carex sylvatica.* The significant increases occurred within a similar magnitude of 31% for *Ficaria verna* and 30% for *Hyacinthoides non-scripta* (Table A4.1.2).

Response of mean traits to environmental treatments

In the second analysis, we tested whether two-way interactive effects of enhanced light availability (L), nitrogen addition (N), warming (T), legacies of prior agriculture (LU) and a covariate (“Soil type”) directly explain variation in mean plant height and mean SLA within species (Figure 4.2).

Plant height increased in response to the environmental treatments and agricultural land-use history across several species ($p < 0.007$, see Table A4.1.3 for p-values) The pattern in significant responses (Figure 4.2A) shows that a total of six species had significant plant height responses (excluding the Soil type covariate), which mostly occurred in slow-colonising species (5 species), compared to fast-colonising species (1 species). However, the covariate “Soil type” significantly affected plant height in three species, all relatively fast colonisers based on CCI. The most important significant ($p < 0.007$) main effect in the plant height response was that of light addition which increased plant height for four species (Figure 4.3A, Table A4.1.4). The largest relative increase in mean plant height to light was found in *Urtica dioica* (+65%) followed by *Carex sylvatica* (+40%), *Anemone nemorosa* (+28%) and *Hyacinthoides non-scripta* (+19%). Nitrogen addition did not affect any species while warming and agricultural land-use history led to one significant response each. *Galium odoratum* responded positively to agricultural land-use history with a relative increase in mean plant height of 15% (Figure 4.3B and Table

A4.1.4). *Vinca minor* responded positively to warming as a main effect with a relative increase of 2% (Figure 4.3C, Table A4.1.4). However, the warming effect depended on light addition; when added together, its height increased by 33%. This was the only interaction that we found in the analysis across all considered species (Figure 4.3D, Table A4.1.4).

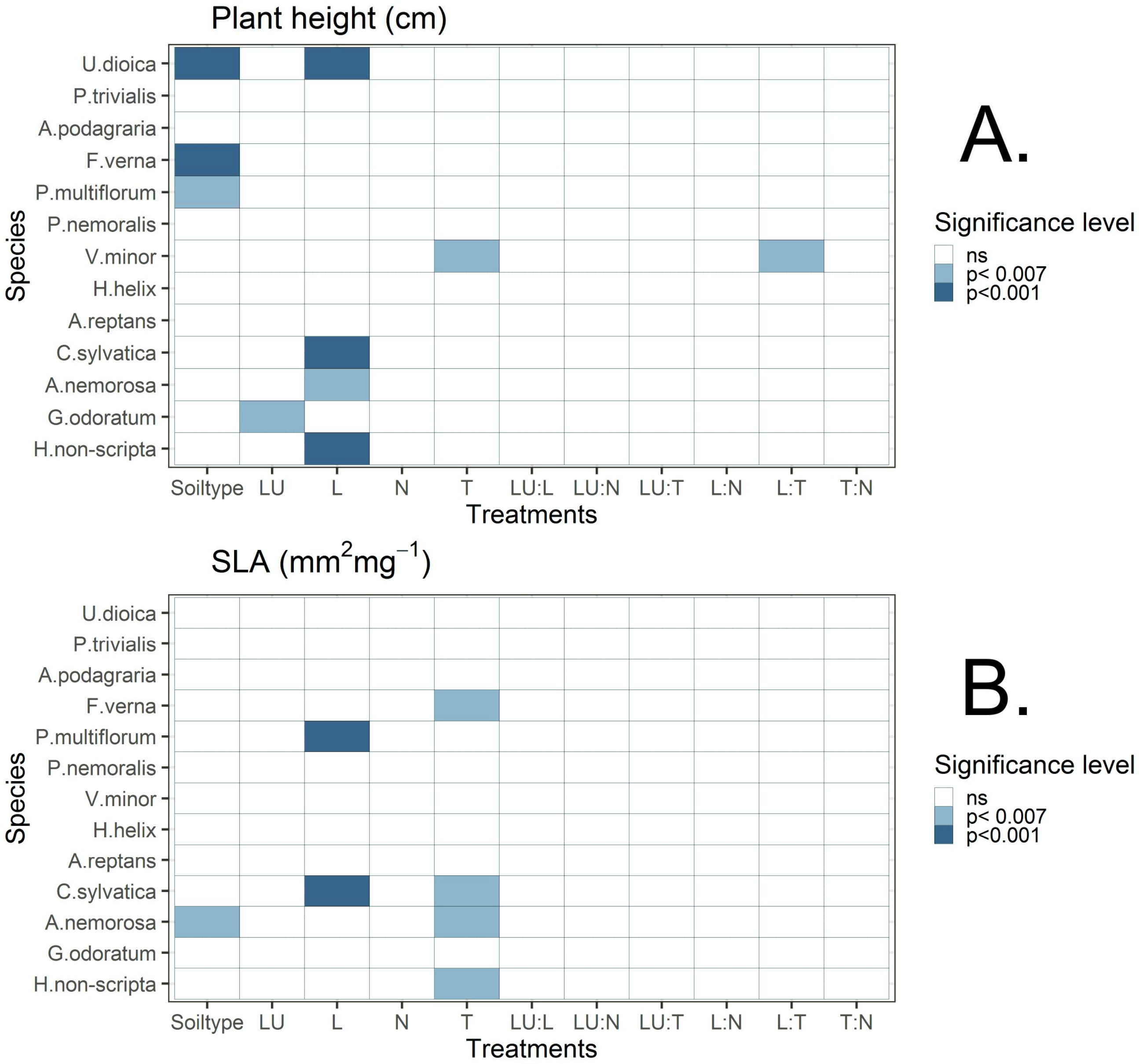


Figure 4.2. Species selectively respond to environmental change in both plant height (panel A) and specific leaf area (panel B). Results of the ANOVA (p<0.007) testing two-way interactions of the forest's land-use history (LU), light availability (L), nitrogen addition (N) and warming (T). We applied an adjusted alpha (p<0.007) so that the expected value of a false positive effect is less than 1 out of the 143 tested terms (alpha = 1/143 = 0.007). Species are ordered on colonisation capacity index (CCI) with the fastest colonisers into post-agricultural forest on top.

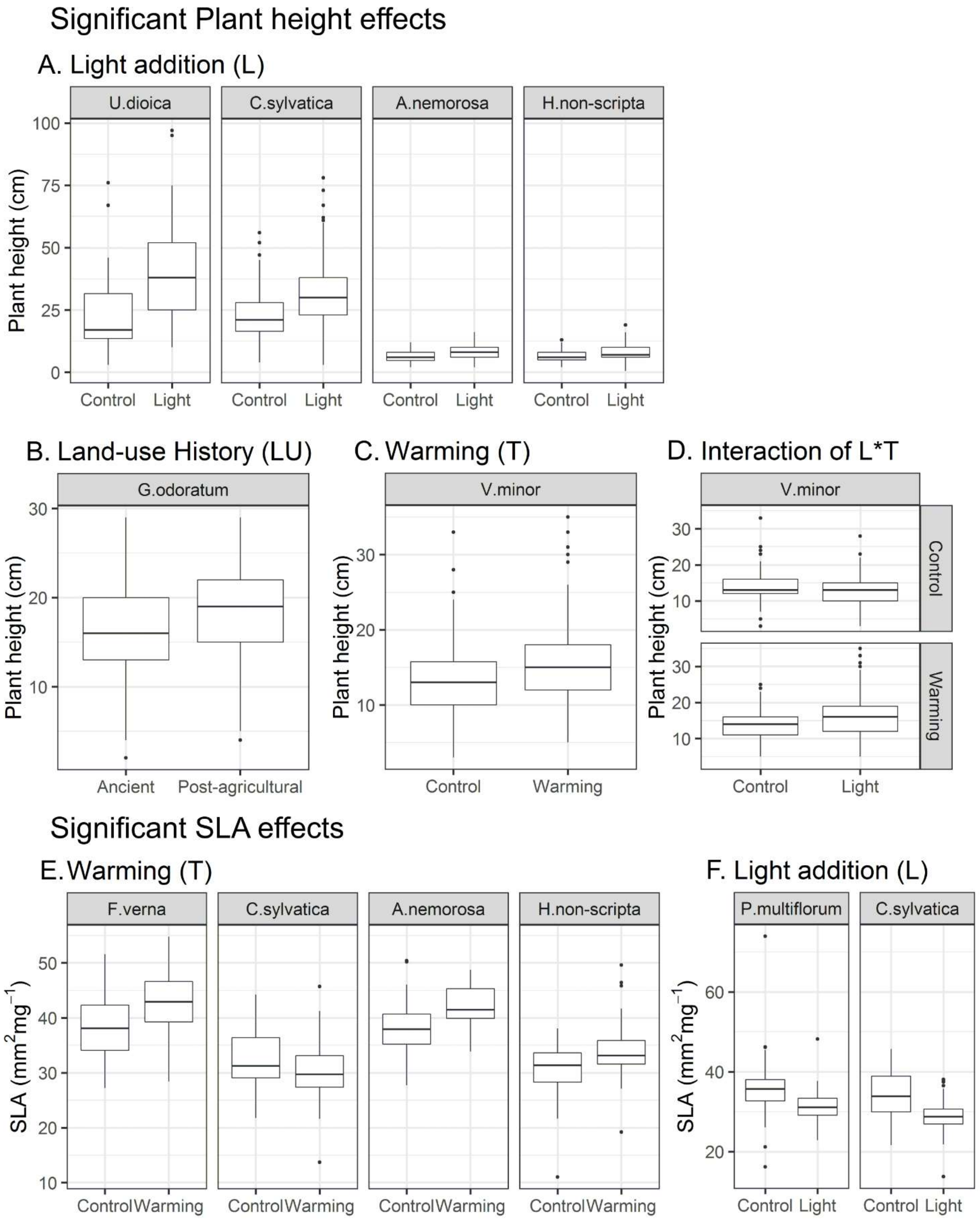


Figure 4.3. Significant effects (p<0.007) of the experimental treatments on within-species plant height (A-D) and SLA (E-F). Panels are illustrations to the significant effects shown in Figure 4.2. Species are ordered on colonisation capacity index (CCI) with faster colonisers on the left. See Table A4.1.5. for absolute trait values of these species.

Specific leaf area (SLA) had less significant responses to the environmental treatments and agricultural land use than plant height (Figure 4.2B). The most important significant (p<0.007) main effect was of warming (Table A4.1.3 for p-values). Warming increased SLA of

Hyacinthoides non-scripta by 11%, by 9 % for *Anemone nemorosa* and by 10% for *Ficaria verna*. Warming negatively affected SLA of *Carex sylvatica* with a decrease of 6% (Figure 4.3E, Table A4.1.4). Light addition had a significant negative main effect for two species: *Carex sylvatica* with a 15% decrease and *Polygonatum multiflorum* with a 10% decrease (Figure 4.3F, Table A4.1.4). SLA within species did not respond to N addition and the forest land-use history and there were no significant interactions. In summary, SLA increased in response to warming in the early flowering species *Hyacinthoides non-scripta*, *Anemone nemorosa* and *Ficaria verna*. Conversely, SLA decreased in late-flowering species, in response to both light and warming (separately) for *Carex sylvatica*.

ITV in relation to a species' colonisation capacity

In the final analysis, we tested whether species' ability to colonise post-agricultural forest correlated with intraspecific variation in plant height and SLA across all treatments (Figure 4.4).

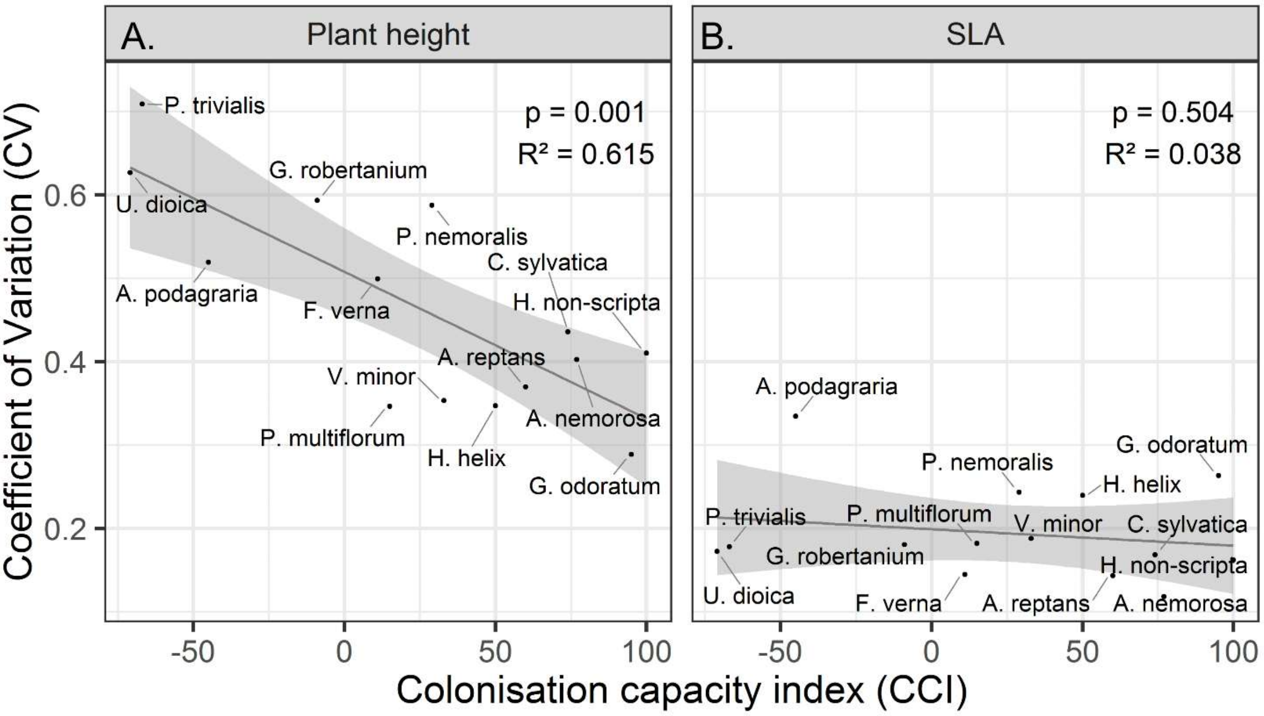


Figure 4.4. Better colonisers into post-agricultural forest exhibit significantly higher ITV, for plant height (panel A) but not SLA (panel B). Coefficient of variation for plant height (panel A) and specific leaf area (panel B) for each species in regression (95% CI) to that species' colonisation capacity index (CCI). CCI values closer to 100 indicate higher affinity for ancient forest; values closer to -100 indicate a faster colonisation into post-agricultural forest (Verheyen et al., 2003).

We found that the coefficient of variation (CV) for plant height was highest ($p = 0.001$, $R^2 = 0.61$) for species that exhibit fast colonisation into post-agricultural forest (negative values in the colonisation capacity index, CCI). CV for plant height ranged from 0.29 for the slow colonising *Galium odoratum* to 0.71 for the fast colonising *Poa trivialis* (Figure 4.4A). CV for SLA ranged from 0.12 for the slow colonising *Anemone nemorosa* to 0.33 for the fast-colonising *Aegopodium* *podagraria* (Figure 4.4B),but this response of SLA to the species' colonisation capacity index was statistically not significant ($p = 0.50$, $R^2 = 0.038$). Fast colonisers into post-agricultural forest thus show more total intraspecific variation in plant height as measured by CV than slow colonisers across the whole trait range, but not in SLA.

Discussion

We found that variation in plant height and SLA of forest herbs under environmental change related to the trait's function. A general observation is that trait values of most species did not respond to more than one main effect of a treatment. Interactions between the multiple environmental treatments were hardly important in explaining variation in mean trait values of species. However, generalizable patterns in plant height and SLA responses arose across species. Mean plant height values increased, as vegetation cover increased and became denser in response to light addition, warming and legacies of prior agriculture. The coefficient of variation (CV) for plant height was larger in fast colonising species. Contrastingly, changes in mean SLA only occurred within selected species to improve light acquisition in shaded conditions. These individualistic, functional trait responses to single factors benefit a particular species' growth in the short term (Garnier, Navas, et al., 2016b). Our results suggest that 1) variation in plant height relates to a greater vegetation cover in bright conditions and largely benefits fast colonisers, and 2) changing SLA is beneficial for species that have acclimatised to shaded conditions in forests, i.e. vernal geophytes and shade-tolerant species.

Intraspecific variation in plant height

The variability in plant height could benefit growth, light acquisition and even dispersal (Moles et al., 2009). Plant size differences between individuals in communities can be an outcome of asymmetric competition for light (DeMalach et al., 2016) as individuals invest in height to disproportionately capture light from neighbours when vegetation becomes denser (Freckleton & Watkinson, 2001). We observed that mean vegetation cover of the community increased from 40% to 75 % due to combined effects of primarily light addition, followed by warming and an agricultural land-use history. As the vegetation became denser and increased to maximal cover, individuals of 10 out of 13 species nearly doubled in mean plant height. This plant height response to vegetation cover was stronger than the plant height response of species to environmental change directly. This observation could indicate that competition mediated by larger vegetation cover better explains plant height change than altered environmental conditions per se. A large meta-analysis on functional trait variation in forest herbs also found that changing vegetation cover, due to environmental change or otherwise, was a strong predictor for larger plant height values in forests herbs (Burton et al., 2017).

Light was the main driver for mean plant height increases and affected four out of thirteen species. Lemke *et al.* (2015) also found that plant height increased in response to light availability, but only in a graminoid and not in a forb. Warming was of minor importance and affected one out of thirteen species. We had expected that more plant species would respond positively to warming, given that individuals of forest herb species are taller in warmer areas of their range (De Frenne et al., 2014; De Frenne, Graae, et al., 2011; Kollmann & Bañuelos, 2004; Lemke et al., 2015). Agricultural land-use history and N addition barely affected plant height values, but including the inherent soil fertility as a covariate in the analysis did explain mean plant height differences in three species. This suggests that pH – influenced nutrient availability can be an important driver for trait variation as well (Siefert, 2012b). Other studies suggest that nutrient enrichment can increase height of forest herb individuals, with a taller plant community as a result (Baeten, Vanhellemont, De Frenne, De Schrijver, et al., 2010; Tatarko & Knops, 2018). However, many of these studies were performed on individual species growing in pots, rather than in a field community such as in our study. The relatively few responses of plant height to environmental change directly could thus exemplify that community properties (i.e. vegetation cover) determine an individual's short-term response to environmental change (Bennett et al., 2016).

We also found that the variability in plant height values (CV) was larger within species with a good colonisation capacity into post-agricultural forest than within slow colonisers. For fast-colonising wind-dispersers, plasticity in height is important to disperse seed further than neighbouring plants (Thomson, Moles, Auld, & Kingsford, 2011) apart from the benefits it yields in capturing light (Freckleton & Watkinson, 2001). Slow colonising forest herbs would need to invest less in above-ground biomass to disperse, as bud banks are more common for multiplication (Klimešová et al., 2016; Ott et al., 2019). Overall, these findings suggest that variation in plant height largely benefits fast colonisers when vegetation cover is high.

Intraspecific variation in specific leaf area (SLA)

SLA is a functional trait that is related to trade-offs in photosynthetic machinery and the associated leaf economics spectrum (Wright et al., 2004). We observed responses of SLA in selected species that typically perform photosynthesis in shaded temperate forest, while we did not find a clear relation between colonisation capacity and the CV of SLA in a species. SLA responded only to light and warming, and solely in shade-avoiding geophytes or shade-tolerant plants that grow under the closed canopy (De Frenne, Baeten, et al., 2011; Verheyen, Honnay, et al., 2003). Interestingly, within-species SLA can both increase or decrease in response to a gradient going from shady to sunny conditions (Garnier, Navas, et al., 2016b). We observed both directions in our study.

Growing temperatures can positively affect SLA of individuals (Atkin et al. 2006). This relationship for forest herbs has been established along latitudinal (De Frenne, Graae, et al. 2011) and elevation gradients (Midolo et al. 2019), where lower SLA is expected at colder, higher latitudes and altitudes. The increasing mean SLA in response to warming of the early flowering geophytes *Hyacinthoides non-scripta*, *Anemone nemorosa* and *Ficaria verna* would thus be advantageous for these spring-flowering geophytes. These are the three earliest species in our species pool to reach their peak biomass during the growing season, and need to withstand frosty nights in early spring to sustain growth. A common response to low air temperatures in plants is to have thicker leaves, which raises leaf mass (Pérez-Harguindeguy et al., 2013) but invokes an important mass-investment trade-off considering the short life-span of their leaves (Jagodziński et al., 2016; Wright et al., 2004). Our three vernal species likely benefitted from the 1°C increase in air temperature because they decreased leaf thickness, and thus mass investments, which yields a higher photosynthetic surface area per unit of mass investment (SLA). Conversely, we found that SLA decreased in response to light for *Carex sylvatica* and *Polygonatum multiflorum*, shade-tolerant species that flower under closed canopies in ancient forests (Bossuyt, Hermy, & Deckers, 1999; Wulf, 1997). Shade tolerant species are generally not plastic in leaf physiology, but can be in leaf morphology (Milla & Reich, 2007; Valladares & Niinemets, 2008; Wright et al., 2004). Individual plants usually develop a higher SLA when grown under low light conditions (Feng & Van Kleunen, 2014; Liu et al., 2016; Reich et al., 2003; Rozendaal et al., 2006) to optimize light capture and carbon gain in those environments (Evans & Poorter, 2001). A lower SLA of the shade-tolerant species *Carex sylvatica* and *Polygonatum multiflorum* within the light treatment followed this pattern.

We found limited effects of N addition and agricultural land-use history on SLA of species. Other studies suggest that intraspecific SLA either has a limited or positive response to enhanced nutrient availability due to land-use legacies (Siefert, 2012b; Siefert & Ritchie, 2016) or N enrichment (Firn et al., 2019; Zhou et al., 2018). In any case, leaf nutrient content and leaf dry matter are more consistent in predicting leaf trait variation in response to nutrient enrichment (Firn et al., 2019; . SLA of multiple forests herbs have been found to be too sensitive to changing light environments to be a reliable predictor of altered nutrient availability (Burton et al., 2017).

Perspectives on trait variation in a changing world

Our experimental design allows disentangling of multiple environmental change treatments on trait expressions of typical forest herb species. A major outcome of this experiment is the lack of treatment interactions on intraspecific trait values. Instead, simple univariate treatment responses of height and SLA were detected. Patterns in plant species growth responses to warming (Hollister et al., 2005), light availability (Rozendaal et al., 2006) and nutrient enrichment (Fynn & O’Connor, 2005) have long been shown to be dependent on the species, suggesting different limiting factors for plant species that naturally occur in the same communities (Chapin & Shaver, 1985). Such selective plant responses to our considered environmental factors imply a “Gleasonian” perspective regarding plant responses to global change and its effect on community organisation: one where individualistic trait expressions to simple environmental factors determines the interactions between species (Gleason, 1926; Götzenberger et al., 2012; Nicolson & Mcintosh, 2002). This individualistic perspective could however pose a gross underestimation of the complex multidimensional interactions between energy and nutrients, consumers and competitors, and time, in affecting plasticity of traits in observed communities (Westneat, Potts, Sasser, & Shaffer, 2019). Our experimental approach did allow us to unravel the importance of drivers that are entangled in the natural world. Important entangled factors are light availability, warming and evapotranspiration in forest gap conditions, and species composition of understorey communities due to legacies of past land use. This disentangled view showed that vegetation cover responded to environmental change and in turn affected trait values of species. Individualistic species responses further promoted ITV due to changing resources and conditions.

Apart from competition in the community affecting ITV (Bennett et al., 2016), species richness effects on ITV could occur as well as a result of resource partitioning (Lipowsky et al., 2015). We did not look into these effects by keeping a constant species richness across communities at the time of planting (5 species); this was not to inflate the experimental design. We also did not look into genetic variation within species, as the component of genetic variation and local adaptation in ITV generally becomes more prominent at larger geographical scales (Albert et al., 2011; Lajoie & Vellend, 2015; Moran et al., 2016; Veresoglou & Penuelas, 2019). However, it is likely that local adaptation in species across a large geographical gradient could alter various trade-offs between functional traits within those species (Oldfather, 2019). Such genetic variation between populations of species could consequently influence the magnitude of ITV in response to environmental change (Kahl, Lenhard, & Joshi, 2019).

Against our expectations, this short-term mesocosm experiment barely revealed any interactive effects between the multiple treatments on plant trait variation. This lack of interaction on the short-term does not imply that such interactive effects are not important for functional trait responses to global change. It rather shows the complementary of experimental research to long-term vegetation resurveys (Perring, Bernhardt-Römermann, et al., 2018; Perring, Diekmann, et al., 2018; G. Verstraeten et al., 2013) or mechanistic modelling approaches (Dirnböck et al., 2017; Landuyt et al., 2018). Long-term experiments, vegetation resurveys and modelling are perhaps better suited to unravel such long-term interactive effects between global change drivers on understorey trait variation (Luo et al., 2011; Verheyen et al., 2017). Most probably, interactions between the treatments in this experiment could emerge after prolonged time when resources and conditions other than light become more limiting (Oliver & Morecroft, 2014).

Conclusions and future directions

It is known that estimating ITV in response to land-use legacies (Siefert & Ritchie, 2016) and environmental change (Albert et al., 2011; Bolnick et al., 2011) can be important for predicting the future of plant communities. Here we assessed ITV in plant height and SLA of forest herbs with different colonisation abilities and affinity to forest habitat, in response to community growth and multiple environmental changes, and on a distinct local scale via a manipulative experiment. Contrary to our expectations, we found that interactions between environmental drivers were not important in explaining variation in traits. A lack of interactive treatment effects could suggest that traits respond individualistically to single factors that benefit a species' growth. We showed that increases in within-species mean plant height relate to a greater vegetation cover in bright conditions, and largely benefitted fast colonisers. Trait variation in SLA promoted species that are acclimated to shaded conditions in forests, as within-species mean SLA distinguished between shade-avoiding vernal species, given their response to warming, and shade-tolerant species, given their response to light addition.

The responses in plant height and SLA occurred after one growing season following herb layer introduction in the experimental communities. With decades of time after the initial trait response (Gross et al., 2009), these patterns could reveal how community assembly veers towards fast-growing and fast-colonising species in disturbed forests when light availability is high. When typical forest herbs would be introduced in such disturbed forests, these could be excluded from community assembly in the long run by more generalist species (see Baeten and Verheyen, 2017). Patterns in ITV at initial stages of plant species establishment could thus potentially reflect the trajectory in community assembly after prolonged time

Acknowledgements

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Appendix 4.1. Additional statistical analyses

Table A4.1.1. Number of trait measurements within each combination of temperature (T), Light addition (L) , N addition (N) and the land-use history of the soil (Ancient vs Post-agricultural). A letter in the rows for Temperature, Light and Nitrogen indicates the application of that treatment. The remainder of the table is made up of measurements for plant height (maximum = 512 per species), and SLA (maximum = 128 per species). Two species (*G. hederacea* and *G. robertianum*) had limited number of measurements for both traits and were consequently left out of the analysis. The species here are ordered on CCI, with fastest colonisers on top.

Land-use history		**Ancient**								**Post-agricultural**							
Temperature			**T**			**T**	**T**		**T**		**T**			**T**	**T**		**T**
Light				**L**		**L**		**L**	**L**			**L**		**L**		**L**	**L**
Nitrogen					**N**		**N**	**N**	**N**				**N**		**N**	**N**	**N**
Height	**Total**																
U. dioica	82	8	4	4	1	4	3	7	4	4	4	8	3	10	8	5	5
P. trivialis	179	6	8	13	11	14	18	3	16	11	15	9	9	22	16	1	7
A. podagraria	175	10	15	16	13	11	7	5	10	14	3	12	10	4	13	18	14
G. robertanium	50			1	1	5	2	2		4	2	5		11	7		10
G. hederacea	12		1			2		1	1			3					4
F. verna	434	34	19	17	18	35	26	28	26	14	36	43	39	22	23	32	22
P. multiflorum	383	25	24	8	20	22	29	30	21	21	22	45	30	26	21	17	22
P. nemoralis	301	13	12	29	17	28	15	19	20	20	24	6	11	11	30	17	29
V. minor	369	26	15	18	24	30	18	23	20	18	23	25	25	24	22	27	31
H. helix	147	3	9	13	4	7	8	7	24	9	9	15	5	7	6	12	9
A. reptans	99	1	16	11	5	14	6		10	1	2	3		9	10	4	7
C. sylvatica	422	12	33	22	18	19	30	29	36	32	18	38	17	43	19	32	24
A. nemorosa	142	14	13	13	7	20	7	11	2	8	9	5	3	9	7	4	10
G. odoratum	387	30	11	32	28	29	13	17	32	14	29	24	28	25	22	30	23
H. non-scripta	263	11	20	29	21	10	10	19	20	32	9	14	11	14	14	19	10
SLA	**Total**																
U. dioica	47	3	3	2	1	4	3	2	3	2	4	5	1	5	3	3	3
P. trivialis	64	1	2	6	8	5	5	2	5	4	6	2	4	7	5		2
A. podagraria	71	5	7	4	4	4	4	5	5	5	1	4	5	2	5	7	4
G. robertanium	21			1	1	1	1	2		1	1	3		4	2		4
G. hederacea	4							1	1			1					1
F. verna	108	9	5	4	5	8	6	6	7	3	9	10	8	7	6	8	7
P. multiflorum	122	9	8	3	7	7	9	9	7	6	7	13	8	8	7	6	8
P. nemoralis	96	5	3	10	5	9	4	7	6	8	7	3	5	4	8	5	7
V. minor	109	8	5	5	7	8	5	7	6	5	7	6	8	6	8	9	9
H. helix	67	1	5	5	3	4	4	3	8	5	7	5	3	5	2	3	4
A. reptans	37	1	6	3	2	4	4		4	1	1	1		3	3	1	3
C. sylvatica	114	4	9	6	5	5	8	7	9	9	7	9	6	10	6	8	6
A. nemorosa	63	6	3	7	4	9	3	5	1	4	4	3	2	3	3	1	5
G. odoratum	102	9	3	8	6	8	4	4	8	5	7	6	8	7	6	7	6
H. non-scripta	100	4	7	10	8	5	5	5	3	11	5	5	6	7	7	8	4

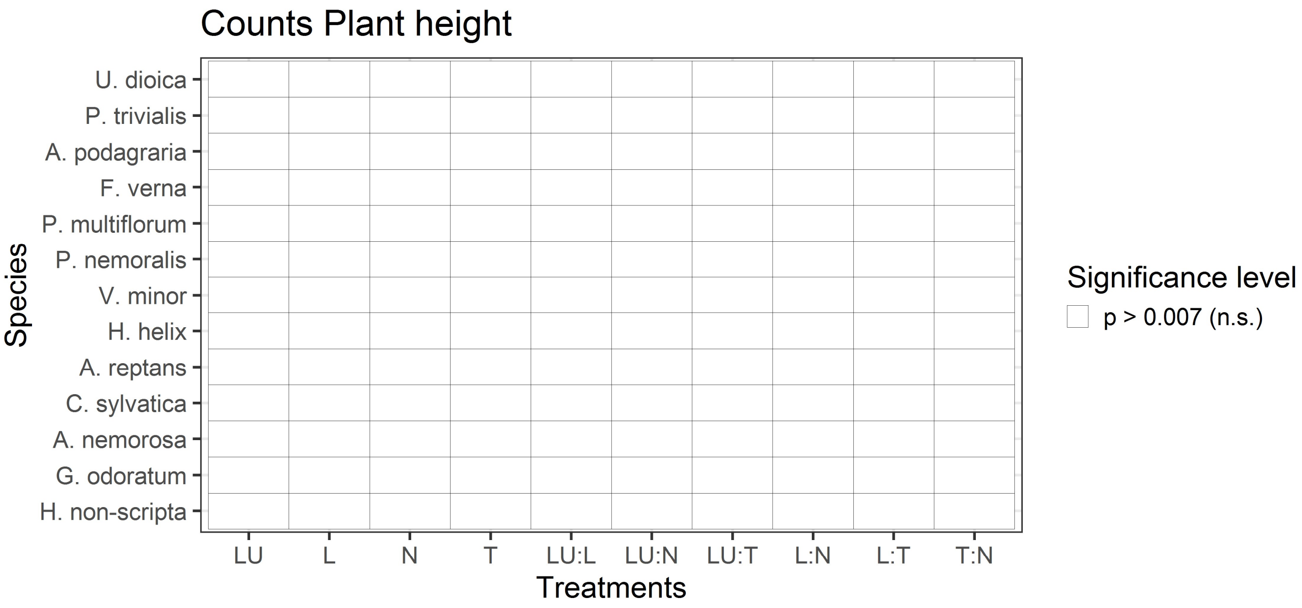

Figure A4.1.1. Number of survivors after one year did not depend on the treatment. The figure shows ANOVA on number of plant height measurements (i.e. survivors) in the replicates of T,L,N,LU ; evaluated with two-way interactions. The p-value threshold in this analysis is the same as for the main analysis of Chapter 4 (see Figure 4.2). *G. robertianum* and *G. hederacea* were left out the analysis due to too few measurements. Species are ordered on CCI, with fastest colonisers on top.

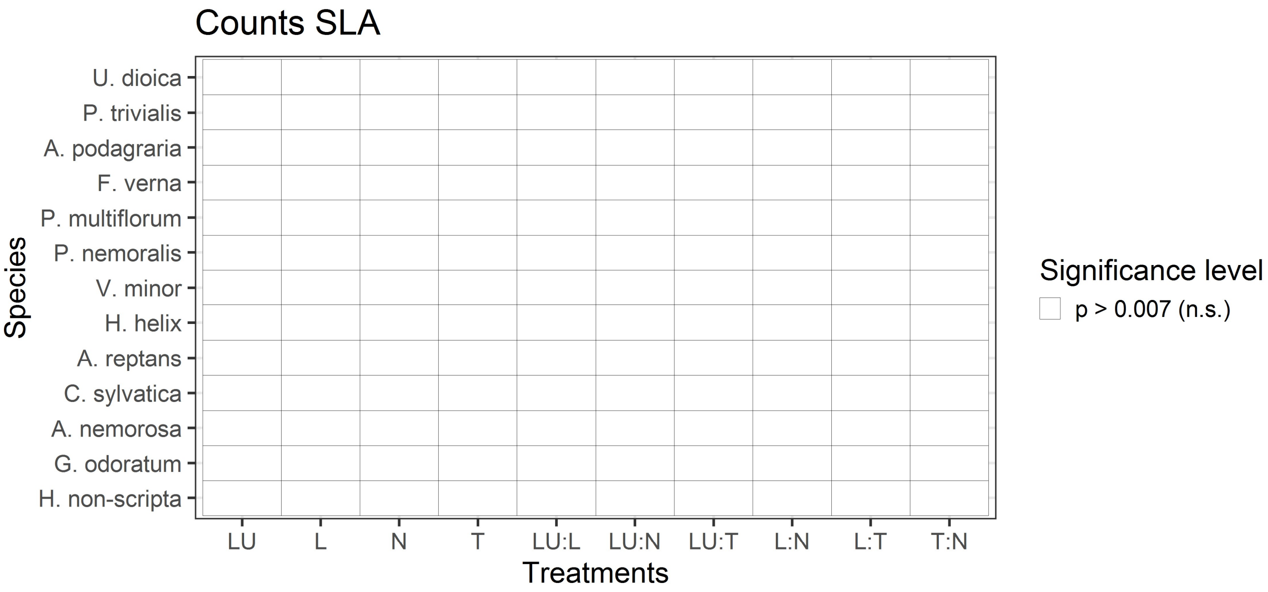

Figure A4.1.2. Number of measurements for SLA did not depend on treatment for any species. The figure shows ANOVA on number of plant height measurements in the replicates of T,L,N,LU ; evaluated with two-way interactions. The p-value threshold of 0.007 in this analysis is the same as for the main analysis (see Figure 3 in main manuscript). *G. robertianum* and *G. hederacea* were left out the analysis due to too few measurements (see Table 1 and Supplementary Table 2). Species are ordered on CCI, with fastest colonisers on top

Table A4.1.2. Coefficients of the species models with total vegetation cover (%) as a predictor, for plant height in cm and SLA in mm^2 mg^{-1}. The lowest (mincover) and largest (maxcover) total vegetation cover values at which a species was recorded are given to denote the range at which they occurred. Denominator degrees of freedom (DF), F test (F.value) and p value (alpha = 0.05) are given in addition to marginal R^2 (R^{2}m) and conditional R^2 (R^{2}c), the latter giving the model fit with fixed and random effects included. Average untransformed intercept and slope values are given, and the distribution of the response variable. Back-transformed predicted value of the models are given, which are the predictions for the trait at lowest cover (min.avg) and at highest cover (max.avg). We calculated the lower and upper 95% confidence intervals for these averages respectively. Note that, for responses that were log-transformed, these intervals are asymmetrical. The relative percentage change was calculated as 100*(max.avg-min.avg)/min.avg, so that an unchanged mean trait value would lead to a zero.

Species	Response	mincover	maxcover	DF	F.value	p.value	R2m	R2c	Distribution	intercept	slope	min.avg	min.lwr	min.upr	max.avg	max.lwr	max.upr	Relative change (%)
U.dioica	Height	36.75	95.84	25	13.68	**0.001**	0.14	0.34	lognormal	2.16	0.02	15.82	8.43	29.68	41.60	22.18	78.03	163
P. trivialis	Height	18.01	95.57	43	21.08	**0.000**	0.11	0.16	lognormal	1.71	0.01	6.91	4.91	9.71	18.03	12.83	25.34	161
A. podagraria	Height	13.65	94.73	46	29.63	**0.000**	0.15	0.15	normal	6.06	0.14	7.91	5.90	9.92	18.92	14.89	22.96	139
F. verna	Height	9.29	97.35	316	76.62	**0.000**	0.32	0.77	normal	1.03	0.10	1.94	1.05	2.83	10.59	8.71	12.47	445
P. multiflorum	Height	21.41	95.75	259	13.11	**0.000**	0.04	0.11	normal	19.96	0.11	22.24	19.55	24.93	30.17	25.29	35.05	36
P.nemoralis	Height	12.62	87.71	194	13.45	**0.000**	0.04	0.30	lognormal	2.59	0.01	14.70	11.67	18.51	26.70	21.20	33.64	82
V. minor	Height	10.62	97.35	258	47.39	**0.000**	0.14	0.21	normal	8.83	0.09	9.75	8.76	10.73	17.22	15.15	19.29	77
H. helix	Height	15.55	94.73	79	0.34	0.561	0.00	0.16	lognormal	2.25	0.00	9.63	7.81	11.87	10.32	8.37	12.72	7
A. reptans	Height	17.01	91.22	60	2.61	0.111	0.04	0.54	lognormal	1.66	0.00	5.71	3.67	8.89	8.27	5.31	12.88	45
C. sylvatica	Height	21.73	94.73	305	62.14	**0.000**	0.17	0.38	lognormal	2.41	0.01	14.39	11.71	17.69	33.85	27.54	41.61	135
A. nemorosa	Height	6.27	87.11	68	12.89	**0.001**	0.08	0.08	normal	5.35	0.05	5.64	4.99	6.29	9.35	7.67	11.04	66
G. odoratum	Height	6.27	97.35	279	169.82	**0.000**	0.41	0.58	normal	6.13	0.16	7.11	6.16	8.07	21.31	19.27	23.36	200
H. non-scripta	Height	5.32	91.22	156	2.37	0.126	0.01	0.21	normal	6.30	0.02	6.38	5.76	7.00	7.73	6.23	9.23	21
U.dioica	SLA	36.75	95.84	21	0.20	0.663	0.00	0.29	normal	89.38	-0.05	87.38	74.00	100.76	84.18	63.55	104.80	-4
P. trivialis	SLA	18.01	95.57	34	1.60	0.215	0.03	0.17	normal	58.31	-0.08	56.81	51.45	62.17	50.34	39.86	60.82	-11
A. podagraria	SLA	13.65	94.73	39	6.82	**0.013**	0.09	0.09	lognormal	4.12	0.00	58.05	46.88	71.87	40.90	33.03	50.65	-30
F. verna	SLA	12.42	97.35	75	24.80	**0.000**	0.19	0.19	normal	31.92	0.12	33.44	31.37	35.51	43.83	39.67	47.99	31
P. multiflorum	SLA	21.41	95.75	89	0.43	0.511	0.01	0.09	lognormal	3.54	0.00	33.96	30.03	38.42	32.51	28.74	36.78	-4
P.nemoralis	SLA	12.62	87.71	65	7.00	**0.010**	0.07	0.07	normal	60.30	-0.18	57.96	53.41	62.51	44.08	34.28	53.87	-24
V. minor	SLA	10.62	97.35	76	7.23	**0.009**	0.06	0.06	normal	30.26	-0.06	29.67	28.12	31.21	24.83	21.49	28.18	-16
H. helix	SLA	15.55	94.73	36	0.03	0.863	0.00	0.00	lognormal	3.30	0.00	26.94	22.04	32.94	26.42	21.61	32.30	-2
A. reptans	SLA	17.01	91.22	15	0.39	0.542	0.01	0.01	lognormal	3.47	0.00	32.58	27.38	38.76	34.50	29.00	41.05	6
C. sylvatica	SLA	23.25	94.73	81	56.79	**0.000**	0.33	0.44	normal	42.64	-0.17	38.76	36.67	40.85	26.81	23.14	30.48	-31
A. nemorosa	SLA	5.32	87.32	34	2.36	0.133	0.04	0.19	normal	38.23	0.05	38.48	36.86	40.10	42.31	38.20	46.43	10
G. odoratum	SLA	9.29	97.35	70	2.34	0.131	0.03	0.17	lognormal	3.98	0.00	52.33	43.61	62.79	44.30	36.92	53.16	-15
H. non-scripta	SLA	5.32	91.22	67	17.90	**0.000**	0.16	0.19	normal	26.68	0.11	27.25	25.67	28.82	36.39	32.66	40.13	34

Table A4.1.3. All ANOVA results from 13 species (143 terms). These terms were performed of the model with structure Soiltype+LU*L+LU*T+LU*N+L*T+L*N+T*N + (1|COM)+(1|Region)+(1|Plot). The Plot-level random effect term was not included in the analysis for SLA.

Species	**term**	**D.F.**	**F.value**	**p.value**	**Significance**	**trait**	**Distribution**
H. non-scripta	Soiltype	67	0.851	0.432	ns	Height	normal
H. non-scripta	LU	153	0.059	0.809	ns	Height	normal
H. non-scripta	L	67	12.543	0.001	p<0.001	Height	normal
H. non-scripta	T	67	0.158	0.693	ns	Height	normal
H. non-scripta	N	67	0.012	0.913	ns	Height	normal
H. non-scripta	LU:L	153	0.841	0.361	ns	Height	normal
H. non-scripta	LU:T	153	0.786	0.377	ns	Height	normal
H. non-scripta	LU:N	153	0.690	0.407	ns	Height	normal
H. non-scripta	L:T	67	0.009	0.923	ns	Height	normal
H. non-scripta	L:N	67	0.876	0.353	ns	Height	normal
H. non-scripta	T:N	67	0.958	0.331	ns	Height	normal
G. odoratum	Soiltype	66	4.196	0.019	ns	Height	normal
G. odoratum	LU	277	9.356	0.002	p< 0.007	Height	normal
G. odoratum	L	66	0.555	0.459	ns	Height	normal
G. odoratum	T	66	4.259	0.043	ns	Height	normal
G. odoratum	N	66	0.539	0.466	ns	Height	normal
G. odoratum	LU:L	277	0.062	0.803	ns	Height	normal
G. odoratum	LU:T	66	0.414	0.522	ns	Height	normal
G. odoratum	LU:N	277	1.513	0.220	ns	Height	normal
G. odoratum	L:T	66	3.904	0.052	ns	Height	normal
G. odoratum	L:N	66	0.497	0.483	ns	Height	normal
G. odoratum	T:N	66	0.514	0.476	ns	Height	normal
A. nemorosa	Soiltype	34	0.797	0.459	ns	Height	lognormal
A. nemorosa	LU	66	0.240	0.626	ns	Height	lognormal
A. nemorosa	L	34	11.838	0.002	p< 0.007	Height	lognormal
A. nemorosa	T	34	5.151	0.030	ns	Height	lognormal
A. nemorosa	N	34	0.007	0.935	ns	Height	lognormal
A. nemorosa	LU:L	66	0.441	0.509	ns	Height	lognormal
A. nemorosa	LU:T	66	4.365	0.041	ns	Height	lognormal
A. nemorosa	LU:N	34	0.506	0.482	ns	Height	lognormal
A. nemorosa	L:T	34	0.575	0.453	ns	Height	lognormal
A. nemorosa	L:N	34	2.167	0.150	ns	Height	lognormal
A. nemorosa	T:N	34	0.517	0.477	ns	Height	lognormal
C.sylvatica	Soiltype	76	3.606	0.032	ns	Height	lognormal
C.sylvatica	LU	302	0.724	0.396	ns	Height	lognormal
C.sylvatica	L	76	32.234	0.000	p<0.001	Height	lognormal
C.sylvatica	T	76	0.936	0.336	ns	Height	lognormal
C.sylvatica	N	76	1.953	0.166	ns	Height	lognormal
C.sylvatica	LU:L	302	0.005	0.943	ns	Height	lognormal
C.sylvatica	LU:T	302	0.406	0.525	ns	Height	lognormal
C.sylvatica	LU:N	302	0.518	0.472	ns	Height	lognormal
C.sylvatica	L:T	76	0.024	0.877	ns	Height	lognormal
C.sylvatica	L:N	76	0.219	0.641	ns	Height	lognormal
C.sylvatica	T:N	76	0.229	0.633	ns	Height	lognormal
A. reptans	Soiltype	3	0.329	0.743	ns	Height	normal
A. reptans	LU	3	4.113	0.136	ns	Height	normal
A. reptans	L	3	7.313	0.074	ns	Height	normal
A. reptans	T	3	0.000	0.985	ns	Height	normal
A. reptans	N	3	25.739	0.015	ns	Height	normal
A. reptans	LU:L	3	10.879	0.046	ns	Height	normal
A. reptans	LU:T	3	2.002	0.252	ns	Height	normal
A. reptans	LU:N	3	0.135	0.738	ns	Height	normal
A. reptans	L:T	3	2.145	0.239	ns	Height	normal
A. reptans	L:N	3	4.531	0.123	ns	Height	normal
A. reptans	T:N	3	8.398	0.063	ns	Height	normal
H. helix	Soiltype	27	2.905	0.072	ns	Height	lognormal
H. helix	LU	78	0.894	0.347	ns	Height	lognormal
H. helix	L	27	0.001	0.982	ns	Height	lognormal
H. helix	T	27	0.095	0.761	ns	Height	lognormal
H. helix	N	27	0.622	0.437	ns	Height	lognormal
H. helix	LU:L	27	0.051	0.823	ns	Height	lognormal
H. helix	LU:T	78	5.556	0.021	ns	Height	lognormal
H. helix	LU:N	27	0.273	0.606	ns	Height	lognormal
H. helix	L:T	27	1.576	0.220	ns	Height	lognormal
H. helix	L:N	27	0.002	0.963	ns	Height	lognormal
H. helix	T:N	27	5.569	0.026	ns	Height	lognormal
V. minor	Soiltype	68	3.266	0.044	ns	Height	normal
V. minor	LU	257	3.769	0.053	ns	Height	normal
V. minor	L	68	2.985	0.089	ns	Height	normal
V. minor	T	68	11.814	0.001	p< 0.007	Height	normal
V. minor	N	68	4.897	0.030	ns	Height	normal
V. minor	LU:L	257	0.618	0.433	ns	Height	normal
V. minor	LU:T	68	2.731	0.103	ns	Height	normal
V. minor	LU:N	68	0.001	0.978	ns	Height	normal

Species	term	D.F.	F.value	p.value	Significance	trait	Distribution
V. minor	L:T	68	9.266	0.003	p< 0.007	Height	normal
V. minor	L:N	68	0.103	0.749	ns	Height	normal
V. minor	T:N	68	0.016	0.899	ns	Height	normal
P. nemoralis	Soiltype	64	0.858	0.429	ns	Height	lognormal
P. nemoralis	LU	194	0.016	0.900	ns	Height	lognormal
P. nemoralis	L	64	1.345	0.250	ns	Height	lognormal
P. nemoralis	T	64	0.034	0.853	ns	Height	lognormal
P. nemoralis	N	64	2.482	0.120	ns	Height	lognormal
P. nemoralis	LU:L	64	0.795	0.376	ns	Height	lognormal
P. nemoralis	LU:T	64	1.393	0.242	ns	Height	lognormal
P. nemoralis	LU:N	64	0.028	0.867	ns	Height	lognormal
P. nemoralis	L:T	64	0.571	0.452	ns	Height	lognormal
P. nemoralis	L:N	64	1.542	0.219	ns	Height	lognormal
P. nemoralis	T:N	64	1.470	0.230	ns	Height	lognormal
P. multiflorum	Soiltype	81	6.310	0.003	p< 0.007	Height	normal
P. multiflorum	LU	258	0.478	0.490	ns	Height	normal
P. multiflorum	L	81	3.874	0.052	ns	Height	normal
P. multiflorum	T	81	1.525	0.220	ns	Height	normal
P. multiflorum	N	81	0.013	0.911	ns	Height	normal
P. multiflorum	LU:L	81	3.035	0.085	ns	Height	normal
P. multiflorum	LU:T	258	1.405	0.237	ns	Height	normal
P. multiflorum	LU:N	81	0.081	0.777	ns	Height	normal
P. multiflorum	L:T	81	2.970	0.089	ns	Height	normal
P. multiflorum	L:N	81	0.825	0.366	ns	Height	normal
P. multiflorum	T:N	81	2.437	0.122	ns	Height	normal
F. verna	Soiltype	76	10.308	0.000	p<0.001	Height	normal
F. verna	LU	314	0.574	0.449	ns	Height	normal
F. verna	L	76	0.752	0.389	ns	Height	normal
F. verna	T	76	2.499	0.118	ns	Height	normal
F. verna	N	76	0.025	0.875	ns	Height	normal
F. verna	LU:L	314	1.200	0.274	ns	Height	normal
F. verna	LU:T	76	0.226	0.636	ns	Height	normal
F. verna	LU:N	314	1.963	0.162	ns	Height	normal
F. verna	L:T	76	2.963	0.089	ns	Height	normal
F. verna	L:N	76	2.213	0.141	ns	Height	normal
F. verna	T:N	76	0.263	0.609	ns	Height	normal
A. podagraria	Soiltype	35	3.200	0.053	ns	Height	lognormal
A. podagraria	LU	35	5.999	0.019	ns	Height	lognormal
A. podagraria	L	35	5.615	0.023	ns	Height	lognormal
A. podagraria	T	35	3.072	0.088	ns	Height	lognormal
A. podagraria	N	35	0.301	0.587	ns	Height	lognormal
A. podagraria	LU:L	35	0.234	0.632	ns	Height	lognormal
A. podagraria	LU:T	35	2.931	0.096	ns	Height	lognormal
A. podagraria	LU:N	35	0.326	0.572	ns	Height	lognormal
A. podagraria	L:T	35	0.047	0.830	ns	Height	lognormal
A. podagraria	L:N	35	1.248	0.272	ns	Height	lognormal
A. podagraria	T:N	35	1.356	0.252	ns	Height	lognormal
P. trivialis	Soiltype	32	2.248	0.122	ns	Height	lognormal
P. trivialis	LU	32	2.380	0.133	ns	Height	lognormal
P. trivialis	L	32	6.552	0.015	ns	Height	lognormal
P. trivialis	T	32	3.657	0.065	ns	Height	lognormal
P. trivialis	N	32	6.212	0.018	ns	Height	lognormal
P. trivialis	LU:L	32	4.698	0.038	ns	Height	lognormal
P. trivialis	LU:T	32	0.020	0.888	ns	Height	lognormal
P. trivialis	LU:N	32	0.986	0.328	ns	Height	lognormal
P. trivialis	L:T	32	0.638	0.430	ns	Height	lognormal
P. trivialis	L:N	32	1.926	0.175	ns	Height	lognormal
P. trivialis	T:N	32	4.024	0.053	ns	Height	lognormal
U. dioica	Soiltype	14	12.677	0.001	p<0.001	Height	normal
U. dioica	LU	14	7.832	0.014	ns	Height	normal
U. dioica	L	14	19.222	0.001	p<0.001	Height	normal
U. dioica	T	14	3.071	0.102	ns	Height	normal
U. dioica	N	14	6.853	0.020	ns	Height	normal
U. dioica	LU:L	14	3.817	0.071	ns	Height	normal
U. dioica	LU:T	14	0.668	0.427	ns	Height	normal
U. dioica	LU:N	14	0.248	0.626	ns	Height	normal
U. dioica	L:T	14	0.018	0.896	ns	Height	normal
U. dioica	L:N	14	0.000	0.986	ns	Height	normal
U. dioica	T:N	14	8.754	0.010	ns	Height	normal
H. non-scripta	Soiltype	56	0.697	0.502	ns	SLA	normal
H. non-scripta	LU	56	0.493	0.486	ns	SLA	normal
H. non-scripta	L	56	0.580	0.450	ns	SLA	normal
H. non-scripta	T	56	11.917	0.001	p< 0.007	SLA	normal
H. non-scripta	N	56	0.024	0.878	ns	SLA	normal
H. non-scripta	LU:L	56	0.763	0.386	ns	SLA	normal
H. non-scripta	LU:T	56	0.932	0.339	ns	SLA	normal
H. non-scripta	LU:N	56	0.335	0.565	ns	SLA	normal
H. non-scripta	L:T	56	0.072	0.790	ns	SLA	normal
H. non-scripta	L:N	56	1.059	0.308	ns	SLA	normal

Species	term	D.F.	F.value	p.value	Significance	trait	Distribution
H. non-scripta	T:N	56	3.725	0.059	ns	SLA	normal
G. odoratum	Soiltype	59	2.212	0.118	ns	SLA	normal
G. odoratum	LU	59	0.081	0.777	ns	SLA	normal
G. odoratum	L	59	6.810	0.011	ns	SLA	normal
G. odoratum	T	59	3.166	0.080	ns	SLA	normal
G. odoratum	N	59	0.317	0.576	ns	SLA	normal
G. odoratum	LU:L	59	0.866	0.356	ns	SLA	normal
G. odoratum	LU:T	59	5.080	0.028	ns	SLA	normal
G. odoratum	LU:N	59	0.007	0.932	ns	SLA	normal
G. odoratum	L:T	59	0.125	0.725	ns	SLA	normal
G. odoratum	L:N	59	0.189	0.665	ns	SLA	normal
G. odoratum	T:N	59	0.011	0.918	ns	SLA	normal
A. nemorosa	Soiltype	23	8.880	0.001	p< 0.007	SLA	normal
A. nemorosa	LU	23	0.860	0.363	ns	SLA	normal
A. nemorosa	L	23	6.246	0.020	ns	SLA	normal
A. nemorosa	T	23	9.405	0.005	p< 0.007	SLA	normal
A. nemorosa	N	23	1.143	0.296	ns	SLA	normal
A. nemorosa	LU:L	23	1.968	0.174	ns	SLA	normal
A. nemorosa	LU:T	23	0.072	0.791	ns	SLA	normal
A. nemorosa	LU:N	23	0.339	0.566	ns	SLA	normal
A. nemorosa	L:T	23	3.027	0.095	ns	SLA	normal
A. nemorosa	L:N	23	1.873	0.184	ns	SLA	normal
A. nemorosa	T:N	23	3.665	0.068	ns	SLA	normal
C.sylvatica	Soiltype	70	1.122	0.331	ns	SLA	normal
C.sylvatica	LU	70	0.177	0.675	ns	SLA	normal
C.sylvatica	L	70	39.432	0.000	p<0.001	SLA	normal
C.sylvatica	T	70	7.873	0.006	p< 0.007	SLA	normal
C.sylvatica	N	70	0.640	0.426	ns	SLA	normal
C.sylvatica	LU:L	70	0.151	0.698	ns	SLA	normal
C.sylvatica	LU:T	70	1.534	0.220	ns	SLA	normal
C.sylvatica	LU:N	70	0.230	0.633	ns	SLA	normal
C.sylvatica	L:T	70	0.198	0.658	ns	SLA	normal
C.sylvatica	L:N	70	0.013	0.911	ns	SLA	normal
C.sylvatica	T:N	70	1.533	0.220	ns	SLA	normal
A. reptans	Soiltype	4	1.396	0.347	ns	SLA	lognormal
A. reptans	LU	4	1.823	0.248	ns	SLA	lognormal
A. reptans	L	4	0.102	0.765	ns	SLA	lognormal
A. reptans	T	4	1.947	0.235	ns	SLA	lognormal
A. reptans	N	4	2.849	0.167	ns	SLA	lognormal
A. reptans	LU:L	4	3.108	0.153	ns	SLA	lognormal
A. reptans	LU:T	4	1.827	0.248	ns	SLA	lognormal
A. reptans	LU:N	4	0.837	0.412	ns	SLA	lognormal
A. reptans	L:T	4	0.000	0.988	ns	SLA	lognormal
A. reptans	L:N	4	2.342	0.201	ns	SLA	lognormal
A. reptans	T:N	4	0.240	0.650	ns	SLA	lognormal
H. helix	Soiltype	25	0.383	0.685	ns	SLA	lognormal
H. helix	LU	25	0.310	0.583	ns	SLA	lognormal
H. helix	L	25	0.346	0.562	ns	SLA	lognormal
H. helix	T	25	0.395	0.535	ns	SLA	lognormal
H. helix	N	25	0.275	0.605	ns	SLA	lognormal
H. helix	LU:L	25	0.001	0.979	ns	SLA	lognormal
H. helix	LU:T	25	0.597	0.447	ns	SLA	lognormal
H. helix	LU:N	25	0.876	0.358	ns	SLA	lognormal
H. helix	L:T	25	0.060	0.808	ns	SLA	lognormal
H. helix	L:N	25	2.951	0.098	ns	SLA	lognormal
H. helix	T:N	25	0.231	0.635	ns	SLA	lognormal
V. minor	Soiltype	65	2.845	0.065	ns	SLA	normal
V. minor	LU	65	0.003	0.954	ns	SLA	normal
V. minor	L	65	1.417	0.238	ns	SLA	normal
V. minor	T	65	4.731	0.033	ns	SLA	normal
V. minor	N	65	4.231	0.044	ns	SLA	normal
V. minor	LU:L	65	0.022	0.883	ns	SLA	normal
V. minor	LU:T	65	1.189	0.279	ns	SLA	normal
V. minor	LU:N	65	1.749	0.191	ns	SLA	normal
V. minor	L:T	65	0.459	0.500	ns	SLA	normal
V. minor	L:N	65	1.502	0.225	ns	SLA	normal
V. minor	T:N	65	0.931	0.338	ns	SLA	normal
P. nemoralis	Soiltype	54	0.558	0.576	ns	SLA	lognormal
P. nemoralis	LU	54	0.068	0.795	ns	SLA	lognormal
P. nemoralis	L	54	1.513	0.224	ns	SLA	lognormal
P. nemoralis	T	54	2.697	0.106	ns	SLA	lognormal
P. nemoralis	N	54	1.831	0.182	ns	SLA	lognormal
P. nemoralis	LU:L	54	0.728	0.397	ns	SLA	lognormal
P. nemoralis	LU:T	54	1.001	0.321	ns	SLA	lognormal
P. nemoralis	LU:N	54	0.422	0.519	ns	SLA	lognormal
P. nemoralis	L:T	54	0.326	0.570	ns	SLA	lognormal
P. nemoralis	L:N	54	0.002	0.968	ns	SLA	lognormal
P. nemoralis	T:N	54	1.298	0.260	ns	SLA	lognormal
P. multiflorum	Soiltype	78	1.736	0.183	ns	SLA	lognormal

Species	term	D.F.	F.value	p.value	Significance	trait	Distribution
P. multiflorum	LU	78	0.383	0.538	ns	SLA	lognormal
P. multiflorum	L	78	13.893	0.000	p<0.001	SLA	lognormal
P. multiflorum	T	78	3.180	0.078	ns	SLA	lognormal
P. multiflorum	N	78	0.024	0.876	ns	SLA	lognormal
P. multiflorum	LU:L	78	0.240	0.626	ns	SLA	lognormal
P. multiflorum	LU:T	78	1.506	0.223	ns	SLA	lognormal
P. multiflorum	LU:N	78	0.771	0.383	ns	SLA	lognormal
P. multiflorum	L:T	78	0.058	0.811	ns	SLA	lognormal
P. multiflorum	L:N	78	0.418	0.520	ns	SLA	lognormal
P. multiflorum	T:N	78	0.012	0.912	ns	SLA	lognormal
F. verna	Soiltype	64	2.632	0.080	ns	SLA	normal
F. verna	LU	64	0.277	0.601	ns	SLA	normal
F. verna	L	64	0.825	0.367	ns	SLA	normal
F. verna	T	64	10.218	0.002	p< 0.007	SLA	normal
F. verna	N	64	0.065	0.799	ns	SLA	normal
F. verna	LU:L	64	1.949	0.167	ns	SLA	normal
F. verna	LU:T	64	2.788	0.100	ns	SLA	normal
F. verna	LU:N	64	0.421	0.519	ns	SLA	normal
F. verna	L:T	64	2.256	0.138	ns	SLA	normal
F. verna	L:N	64	1.101	0.298	ns	SLA	normal
F. verna	T:N	64	0.000	0.998	ns	SLA	normal
A. podagraria	Soiltype	28	0.168	0.846	ns	SLA	lognormal
A. podagraria	LU	28	1.327	0.259	ns	SLA	lognormal
A. podagraria	L	28	1.050	0.314	ns	SLA	lognormal
A. podagraria	T	28	4.729	0.038	ns	SLA	lognormal
A. podagraria	N	28	0.105	0.749	ns	SLA	lognormal
A. podagraria	LU:L	28	0.618	0.439	ns	SLA	lognormal
A. podagraria	LU:T	28	1.470	0.235	ns	SLA	lognormal
A. podagraria	LU:N	28	0.000	0.994	ns	SLA	lognormal
A. podagraria	L:T	28	0.147	0.704	ns	SLA	lognormal
A. podagraria	L:N	28	0.037	0.850	ns	SLA	lognormal
A. podagraria	T:N	28	1.199	0.283	ns	SLA	lognormal
P. trivialis	Soiltype	23	2.192	0.134	ns	SLA	lognormal
P. trivialis	LU	23	5.318	0.030	ns	SLA	lognormal
P. trivialis	L	23	0.459	0.505	ns	SLA	lognormal
P. trivialis	T	23	4.393	0.047	ns	SLA	lognormal
P. trivialis	N	23	0.012	0.914	ns	SLA	lognormal
P. trivialis	LU:L	23	0.049	0.826	ns	SLA	lognormal
P. trivialis	LU:T	23	0.305	0.586	ns	SLA	lognormal
P. trivialis	LU:N	23	0.000	0.988	ns	SLA	lognormal
P. trivialis	L:T	23	0.008	0.929	ns	SLA	lognormal
P. trivialis	L:N	23	2.136	0.157	ns	SLA	lognormal
P. trivialis	T:N	23	0.018	0.895	ns	SLA	lognormal
U. dioica	Soiltype	10	0.090	0.915	ns	SLA	normal
U. dioica	LU	10	1.512	0.247	ns	SLA	normal
U. dioica	L	10	2.300	0.160	ns	SLA	normal
U. dioica	T	10	4.590	0.058	ns	SLA	normal
U. dioica	N	10	1.849	0.204	ns	SLA	normal
U. dioica	LU:L	10	1.677	0.224	ns	SLA	normal
U. dioica	LU:T	10	1.099	0.319	ns	SLA	normal
U. dioica	LU:N	10	0.137	0.719	ns	SLA	normal
U. dioica	L:T	10	0.049	0.829	ns	SLA	normal
U. dioica	L:N	10	2.812	0.124	ns	SLA	normal
U. dioica	T:N	10	0.001	0.972	ns	SLA	normal

Table A4.1.4. Fitted models (t-test) for each species on both plant height and SLA which include only the significant terms that were tested in the full ANOVA ($p<0.007$). We report on estimates (“Est.” and standard errors (“std”) with marginal R^2 (fixed effects), conditional R^2 (full model fit including random effect terms) and f-tests with p-values. Units are cm for plant height and $mm^2\ mg^{-1}$ for SLA. When a response variable was transformed with a natural logarithm (see column “Dist”), this becomes log(cm) or log(mm^2/mg) respectively. We give back-transformed means in the original unit on a 95% confidence interval in columns “Low CI”, “mean” and “high CI” respectively. Intercept values are “Ancient” for Land-use history, “Eutrophic” for Soil type and control for light, warming and Nitrogen addition. The final column gives the size of an effect relative to the intercept value.

Species	trait	Dist	term	Est.	std	Low CI	mean	high CI	R2m	R2c	DF	t-value	P-value	Relative response
H. non-scripta	Height	normal	Intercept	6.47	0.29	5.9	6.5	7.0	0.05	0.22	157	1129	0	
H. non-scripta	Height	normal	L	1.25	0.4	6.9	7.7	8.5	0.05	0.22	74	10	0.003	**19%**
G. odoratum	Height	normal	Intercept	15.6	0.64	14.3	15.6	16.9	0.05	0.62	279	947	0	
G. odoratum	Height	normal	Post-ag	2.32	0.68	16.6	17.9	19.3	0.05	0.62	279	12	0.001	**15%**
A. nemorosa	Height	log	Intercept	1.76	0.05	5.3	5.8	6.4	0.08	0.14	69	2544	0	
A. nemorosa	Height	log	L	0.25	0.07	6.5	7.5	8.6	0.08	0.14	42	11	0.002	**28%**
C.sylvatica	Height	log	Intercept	3	0.05	18.2	20.1	22.2	0.12	0.43	306	7762	0	
C.sylvatica	Height	log	L	0.34	0.06	25.1	28.2	31.7	0.12	0.43	83	32	0	**40%**
V. minor	Height	normal	Intercept	13.54	0.63	12.3	13.5	14.8	0.07	0.2	259	1388	0	
V. minor	Height	normal	L	-0.93	0.83	11.0	12.6	14.2	0.07	0.2	75	1	0.269	**-7%**
V. minor	Height	normal	T	0.31	0.86	12.2	13.9	15.5	0.07	0.2	75	12	0.001	**2%**
V. minor	Height	normal	T:L	3.19	1.17	14.4	16.7	19.0	0.07	0.2	75	7	0.008	**33%**
P. multiflorum	Height	normal	Intercept	30.22	1.29	27.7	30.2	32.7	0.03	0.11	260	2789	0	
P. multiflorum	Height	normal	Meso	-2.24	1.54	25.0	28.0	31.0	0.03	0.11	89	6	0.005	**-7%**
P. multiflorum	Height	normal	Oligo	-4.69	1.49	22.6	25.5	28.5	0.03	0.11	89	6	0.005	**-16%**
F. verna	Height	normal	Intercept	8.72	0.84	7.1	8.7	10.4	0.13	0.77	317	502	0	
F. verna	Height	normal	Meso	-0.11	0.97	6.7	8.6	10.5	0.13	0.77	83	10	0	**-1%**
F. verna	Height	normal	Oligo	-2.87	0.96	4.0	5.9	7.7	0.13	0.77	83	10	0	**-33%**
U. dioica	Height	normal	Intercept	25.27	5.91	13.7	25.3	36.9	0.32	0.41	30	262	0	
U. dioica	Height	normal	L	16.5	4.24	33.5	41.8	21.9	0.32	0.41	23	19	0	**65%**
U. dioica	Height	normal	Meso	7.08	6	20.6	32.4	35.3	0.32	0.41	23	7	0.005	**28%**
U. dioica	Height	normal	Oligo	-9.52	5.95	4.1	15.8	27.4	0.32	0.41	23	7	0.005	**-38%**
H. non-scripta	SLA	normal	Intercept	30.64	0.66	29.3	30.6	31.9	0.1	0.1	67	4177	0	
H. non-scripta	SLA	normal	T	3.36	1	32.0	34.0	36.0	0.1	0.1	67	11	0.001	**11%**
A. nemorosa	SLA	normal	Intercept	38.55	1.81	35.0	38.6	42.1	0.33	0.53	32	4299	0	
A. nemorosa	SLA	normal	Meso	-2.13	1.78	32.9	36.4	39.9	0.33	0.53	32	7	0.002	**-6%**
A. nemorosa	SLA	normal	Oligo	2.13	1.85	37.1	40.7	44.3	0.33	0.53	32	7	0.002	**6%**
A. nemorosa	SLA	normal	T	3.3	1	39.9	41.9	43.8	0.33	0.53	32	13	0.001	**9%**
C.sylvatica	SLA	normal	Intercept	35.32	0.91	33.5	35.3	37.1	0.28	0.43	80	2175	0	
C.sylvatica	SLA	normal	L	-5.17	0.79	28.6	30.2	31.7	0.28	0.43	80	42	0	**-15%**
C.sylvatica	SLA	normal	T	-2.27	0.81	31.5	33.1	34.6	0.28	0.43	80	8	0.006	**-6%**
P. multiflorum	SLA	log	Intercept	3.55	0.02	33.5	34.8	36.2	0.11	0.16	89	55721	0	
P. multiflorum	SLA	log	L	-0.11	0.03	29.4	31.2	33.1	0.11	0.16	89	14	0	**-10%**
F. verna	SLA	normal	Intercept	38.43	0.76	36.9	38.4	39.9	0.11	0.11	75	5694	0	
F. verna	SLA	normal	T	3.76	1.07	40.1	42.2	44.3	0.11	0.11	75	12	0.001	**10%**

Table A4.1.5. Mean Plant height (m) and SLA (mm^2 mg^{-1}) of the 15 species in the experiment. The outer left is the overall population mean, which is used later in Chapter 6 for the community weighted mean value of a “single-species sigle-trait” approach.. An average trait value of each species was calculated within each combination of the two-level treatments of land-use history (ancient vs. post-agricultural), N addition (N), light addition (L) and temperature (T), leading to 16 possible trait values depending on these conditions.

Land-use history		**Ancient**								**Post-agricultural**							
Nitrogen			**N**		**N**		**N**		**N**		**N**		**N**		**N**		**N**
Light				**L**	**L**			**L**	**L**			**L**	**L**			**L**	**L**
Temperature						**T**	**T**	**T**	**T**					**T**	**T**	**T**	**T**
height	mean																
A. podagraria	0.15	0.11	0.14	0.10	0.17	0.13	0.14	0.14	0.18	0.14	0.17	0.14	0.16	0.18	0.16	0.15	0.20
A.reptans	0.08	0.04	0.06	0.11		0.06	0.08	0.09	0.09	0.07	0.05		0.09	0.10	0.07	0.12	0.08
A. nemorosa	0.07	0.06	0.08	0.06	0.07	0.06	0.08	0.06	0.08	0.06	0.07	0.06	0.06	0.07	0.09	0.08	0.09
C. sylvatica	0.28	0.19	0.26	0.20	0.32	0.24	0.31	0.24	0.30	0.22	0.31	0.27	0.31	0.22	0.31	0.22	0.39
F. verna	0.08	0.06	0.05	0.06	0.07	0.07	0.08	0.06	0.09	0.09	0.08	0.07	0.08	0.09	0.09	0.07	0.10
G.odoratum	0.17	0.17	0.13	0.18	0.15	0.13	0.17	0.15	0.20	0.17	0.20	0.17	0.18	0.19	0.18	0.19	0.19
G.robertianum	0.15		0.49	0.10	0.09		0.14	0.12		0.11	0.21			0.18	0.14	0.18	0.14
G.hederacea	0.16				0.10	0.09	0.11		0.19		0.20						0.17
H. helix	0.11	0.11	0.09	0.12	0.10	0.10	0.13	0.10	0.11	0.10	0.10	0.14	0.11	0.11	0.10	0.08	0.09
H. non-scripta	0.07	0.07	0.08	0.07	0.08	0.06	0.09	0.06	0.07	0.06	0.08	0.07	0.08	0.07	0.08	0.07	0.07
P. nemoralis	0.23	0.29	0.23	0.25	0.24	0.16	0.22	0.22	0.28	0.19	0.18	0.20	0.30	0.27	0.21	0.21	0.26
P. trivialis	0.14	0.08	0.16	0.07	0.11	0.08	0.12	0.15	0.16	0.09	0.12	0.16	0.05	0.13	0.16	0.19	0.17
P. multiflorum	0.27	0.26	0.25	0.24	0.27	0.28	0.27	0.28	0.28	0.25	0.30	0.23	0.30	0.27	0.27	0.27	0.30
U. dioica	0.34	0.15	0.32	0.29	0.44	0.19	0.35	0.24	0.22	0.25	0.34	0.33	0.70	0.20	0.42	0.28	0.47
V. minor	0.14	0.13	0.12	0.14	0.12	0.12	0.14	0.14	0.16	0.12	0.13	0.15	0.13	0.14	0.17	0.15	0.18
SLA	mean																
A. podagraria	49.72	52.73	59.44	51.10	71.44	46.75	46.87	42.09	43.48	46.84	50.94	52.87	46.05	44.11	50.20	43.90	44.46
A.reptans	34.22	34.08	34.79	40.96		34.08	33.31	31.19	30.64	31.26	42.46		32.49	34.04	40.54	32.99	33.24
A. nemorosa	40.19	38.88	37.59	36.75	37.91	40.80	43.27	38.00	46.77	40.76	42.96	32.88	34.48	40.55	42.70	38.88	45.41
C. sylvatica	31.37	35.38	29.66	35.49	30.16	34.13	29.37	33.43	28.35	35.84	30.41	34.63	29.74	29.83	25.78	35.17	28.72
F. verna	40.35	37.63	34.68	33.70	39.29	42.20	43.77	39.41	44.73	38.94	39.66	42.58	37.64	41.23	41.82	40.30	43.46
G.odoratum	48.45	56.99	52.71	58.42	41.50	43.89	38.44	45.53	46.09	48.22	43.53	47.65	51.69	54.93	42.94	48.37	46.76
G.robertianum	104.96		68.93	84.23	111.34		87.95	107.45		97.62	109.50			116.93	105.93	120.30	106.41
G.hederacea	66.49				93.01				56.97		61.67						54.32
H. helix	27.23	29.16	24.61	28.58	35.47	24.70	30.35	27.40	25.79	29.09	25.11	22.43	28.60	29.52	24.97	21.46	30.23
H. non-scripta	32.09	29.91	32.28	31.81	30.05	35.47	31.42	31.83	36.74	29.93	33.22	27.77	29.68	33.76	31.35	33.84	40.56
P. nemoralis	51.16	45.32	55.91	51.32	51.09	61.65	49.83	46.71	45.12	60.36	45.04	56.33	55.15	51.73	48.73	47.57	44.00
P. trivialis	53.39	54.33	59.77	57.55	52.87	58.43	49.34	54.54	54.13	54.13	51.09	49.91		51.88	50.23	51.32	46.52
P. multiflorum	33.52	33.04	31.05	36.07	30.46	36.66	32.05	33.37	31.19	37.93	29.79	31.73	33.47	34.97	32.21	42.88	32.99
U. dioica	85.62	90.96	86.29	90.79	64.44	90.01	96.27	87.72	90.24	83.75	80.29	93.34	73.53	95.84	81.56	91.31	73.98
V. minor	26.92	24.64	30.54	29.48	30.32	23.41	24.15	28.26	26.83	26.77	27.76	26.94	27.18	24.87	25.64	26.23	28.34

Image: Sofie Verstraelen

Chapter 5

Larger direct than indirect effects of multiple environmental changes on leaf nitrogen of forest herbs

Haben Blondeel, Elyn Remy, Michael P. Perring, Dries Landuyt, Samuel Bodé, Pieter De Frenne, Pascal Boeckx, Kris Verheyen. *Larger direct than indirect effects of multiple environmental changes on leaf nitrogen of forest herbs*. Plant and Soil (in press).

Abstract

Disentangling direct and indirect effects of global change drivers on plant nitrogen (N) uptake in leaves is important for understanding species and community responses in a changing world. We created understorey herb communities on forest soils with and without recent agricultural history. We traced pulse additions of $^{15}NH_4{}^{15}NO_3$ within these mesocosms while applying two-level factorial treatments of N enrichment, warming and illumination. We modelled direct and indirect effects of these treatments on leaf N content and ^{15}N uptake in leaves. Warming and illumination had three times larger direct negative effects on leaf N content per dry mass and percentage leaf N derived from label (Ndfl%) than their indirect negative effects via an increasing community cover. These results imply a tissue dilution of N with increasing growth, in response to environmental change directly and indirectly exacerbated by community cover. We additionally found that interspecific differences in Ndfl% correlated with a species' colonisation capacity and resource acquisition strategy. Global change can directly affect allocation of N into foliage, with simultaneous indirect effects via altered community properties that influence individual plant responses. Predicting the future of plant communities in a changing world requires accounting for such understudied pathways.

Introduction

The extent of abandoned arable land is increasing across the globe since the latter half of the 20th century (Cramer et al., 2008; Waters et al., 2016). Legacies of prior agriculture, particularly nutrient enrichment in the soil, can persist there for decades to centuries (Falkengren-Grerup et al., 2006; Grossmann & Mladenoff, 2008; Leuschner et al., 2014; Yesilonis et al., 2016). These land-use legacies can, combined with other global change drivers, steer important ecosystem patterns and processes such as plant community composition and nitrogen (N) cycling (Perring et al., 2016). In temperate forests, herbaceous communities have a key role in the cycling of N via their foliage and back into the soil organic matter (Gilliam, 2007). Despite this layer only representing about half of the foliar biomass as compared to overstorey trees (Landuyt, De Lombarerde et al. (2019); Gilliam 2007), herbaceous species have higher N assimilation rates than canopy trees (Buchmann et al., 2004) and root in shallower nutrient rich topsoil layers(Jobbágy & Jackson, 2001). This means the leaf litter of herbaceous species is of better quality compared to foliage of woody species, due to leaf traits that promote N uptake (Cornwell et al., 2008; Díaz et al., 2016). Changing N uptake in herbaceous communities due to legacies of prior agriculture and other global change drivers could thus affect forest ecosystem functioning as a whole, and may depend on ecological strategies of species (Gilliam, 2007).

Functional leaf traits determine N uptake of forest herbs and correlate with a species' colonisation capacity and life-history strategy (Verheyen, Honnay, et al., 2003). Leaf traits of species follow a fast-slow resource acquisition continuum (Wright et al., 2004). These leaf traits, such as the leaf area to mass ratio (specific leaf area, SLA) and the leaf N content per dry mass, either promote large N uptake for fast carbon accumulation (resource acquisition), or resource conservation with slow carbon accumulation (Díaz et al., 2016; Reich, 2014; Wright et al., 2004). Species that initially colonise abandoned arable land (post-agricultural forest) occur at the fast end of the resource acquisition continuum and have a “fast” life-history, i.e. high relative growth rates and short lifespans (Beckman et al., 2018; Díaz et al., 2016). Such fast colonisers have leaves that are characterised by a high SLA and high leaf N content per dry mass (Verheyen, Honnay, et al., 2003) to efficiently capture light in bright and nutrient enriched conditions (Evans & Poorter, 2001; Poorter & Evans, 1998).

Contrastingly, on the slow and resource conservative end of the spectrum, we observe species that slowly colonise into post-agricultural forest and are typical of ancient forest (Verheyen, Honnay, et al., 2003). Slow colonisers have resource conservative leaf traits (Díaz et al., 2016) that, between species, are characterised by a low SLA and low leaf N content per dry mass (Verheyen, Honnay, et al., 2003). These resource conservative leaf traits arise from two contrasting life-history strategies that allow competition for light in shaded conditions

(Valladares et al., 2016; Verheyen, Honnay, et al., 2003). First, there are vernal geophytes with relatively thick leaves that emerge under fully lighted conditions in early spring (before tree canopy flush) and senesce when the forest canopy closes in late spring (Mabry et al., 2008). Second, there are shade tolerant species, which keep photosynthetic tissues throughout the growing season under a closed canopy in dark conditions. Intraspecific variation of leaf traits in these species can occur as they are particularly sensitive to sudden changes in light conditions (Liu et al., 2016; Valladares & Niinemets, 2008).

Multiple global change drivers such as climate change, N enrichment from atmospheric deposition and land-use legacies additionally affect foliar N in the herbaceous understorey (Gilliam, 2007). Environmental changes can have direct physiological effects or indirect community effects on foliar N (Manning et al., 2006). N content per dry mass in herbaceous leaves can directly respond to light and warming, and “dilute” (i.e. decrease) due to increases in plant size and carbon acquisition rates (Jarrell & Beverly, 1981; Sardans et al., 2017; Takeuchi et al., 2001; Yin, 2002). Contrastingly, a direct increase in leaf N content per dry mass can occur in response to N enrichment from depositions (Farrer & Suding, 2016; Fraterrigo et al., 2009) or legacies of prior agriculture (Baeten et al., 2011; Siefert & Ritchie, 2016). At the community level, indirect effects can occur as nutrient enrichment in warm and bright conditions could steer community trait distributions towards dominance of species with innate high tissue N and SLA (Gilliam et al., 2016). On the other hand, these environmental drivers could enhance biomass production of the whole community (Kardol et al., 2010) and increase vegetation cover (Muukkonen et al., 2006). Larger vegetation cover raises asymmetric competition for light between individuals, which pushes plants to invest more in vertical growth (DeMalach et al., 2017, 2016; Freckleton & Watkinson, 2001). Environmental change could thus indirectly exacerbate a decline in leaf N content per dry mass of plants via the community biomass production (Craine et al., 2018; Stevens, 2019). Biomass production in forest understoreys is, however, seasonally variable and depends on life-history strategies of species (Jagodziński et al., 2016).

To understand the effects of environmental change on forest herb N uptake, on both a species and community level, we need to determine N uptake independently of species’ preference for either ancient or post-agricultural forest. We therefore mixed forest herbs with different colonisation abilities and life-history strategies to document herb N uptake under multiple environmental changes and on soils with different land-use history. We monitored direct *in situ* added inorganic N uptake in forest herbaceous communities via $^{15}NH_4{}^{15}NO_3$ pulse labelling. We hypothesize that:

1. Warming, light availability, N enrichment and agricultural legacies can affect N allocation to leaves in plants. Changes in plant community dynamics can alter these effects. Enhanced growth of plants could potentially lead to a dilution of tissue N within plant species.
2. The ability of forest herbs to take up the inorganic ^{15}N tracer in leaves correlates with a species' colonisation capacity and its resource acquisition strategy. Fast colonising resource acquisitive species, as characterised by high SLA and leaf N content per dry mass, retrieve more ^{15}N tracer than slow colonising resource conservative species, which are characterised by a low SLA and leaf N content per dry mass.

Materials and Methods

We performed an *in situ* forest mesocosm experiment where we mixed herbaceous species with different colonisation abilities and planted these on soils from both ancient forest (forested before at least 1850) and post-agricultural forest (forested since 1950). We selected 64 mesocosms from the larger field experiment that was set up in the 1.8 ha fenced area in the Aelmoeseneie forest, Belgium (see Chapter 3). These 64 mesocosms had closely matching soil texture and pH to inject with the ^{15}N tracer. This was to limit confounding of plant N uptake with inherent soil fertility and to isolate effects that agricultural history had on the chemistry of the soil samples (see Appendix Table A5.1.1 and Blondeel et al. 2019).

Experimental set-up

We have two soil origins from two northwestern European regions, with two ancient and two post-agricultural forest soils of each region (treatment "LU" with two replicates). The soil provenances are Flanders, northern Belgium (50°58′30″ N, 3°48′16″ E, MAT = 10.1 °C, MAP = 772 mm and N deposition = 22.5 kg N ha^{-1}) and Skåne, southern Sweden (55° 32′ 47″ N, 13° 14′ 28″ E, MAT = 7.7 °C, MAP = 765 mm, N deposition = 12.2 kg N ha^{-1}). Within each of these two regions, we use soil from two adjacent pairs of ancient forest (forested since at least 1850) and post-agricultural forests (forested since 1950) as substrates for the plant communities (treatment land-use history, "LU"). The legacy of prior agriculture in the particular soils was most evident in the higher soil pH (+0.61 pH units ±0.052 standard error (S.E.)), lower soil C:N ratio (-1.77±0.09 S.E.) and a higher soil Olsen-P concentration (+45 mg kg^{-1} soil ±2.64 S.E.) of post-agricultural forest in comparison with ancient forest (Appendix Table A5.1.1).

We compiled a pool of fifteen species commonly found in temperate European forests (Chapter 3) and divided this species pool according to three emergent groups that are linked with a species' colonisation capacity (De Frenne, Baeten, et al., 2011; Verheyen, Honnay, et al., 2003). The species in this pool can be characterised by the colonisation capacity index (CCI), where more negative values indicate a higher capacity to successfully colonise into post-agricultural forests (Verheyen, Honnay, et al., 2003). The continuous CCI scale exists from -100 to +100, where -100 means very fast colonisation into post-agricultural forest, and +100 means that the species can only be found in ancient forest (see Chapter 3 for more information on CCI). At the time of planting, each mesocosm consisted of two poor colonisers, two common colonisers of post-agricultural forest and one fast-colonising nitrophilic species (see Chapter 3 for details on community assembly). These combinations of five species originated from twelve fixed species combinations that were randomly assembled (Chapter 3) and have a balanced distribution in the subsetted 64 mesocosms for this experiment (Appendix Table A5.1.2).

We have applied three two-level environmental treatments in a full-factorial design since April 2016 (Chapter 3). Ongoing treatments consist of (i) nitrogen enrichment; (ii) experimental warming and (iii) enhanced light availability. The first treatment is N enrichment with an additional 50 kg N ha^{-1} yr^{-1} eq., further referred to as treatment “N”. We applied the N treatment as four seasonal pulses per year, each time adding 0.25 L of a 2.01 g L-1 solution of NH_4NO_3 to each mesocosm and rinsing the leaves with 0.25 L of demineralised water. The control mesocosms received 0.5 L of demineralised water during each of the seasonal additions. The second treatment consisted of experimental warming (referred to as treatment “T”) using 75 cm-wide open top chambers (De Frenne et al., 2010). This treatment significantly raised the air temperate between 1°C and 2° C in spring 2017, but with insignificant temperature effects when the forest canopy closed towards the end of May (Chapter 3). The third treatment, light addition (referred to as “L”) adds around 25 µmol m^{-2}s^{-1} PAR to the ambient light conditions (below 10 µmol m^{-2}s^{-1} under fully closed canopy) by use of two 18 W fluorescent tubes suspended 75 cm above ground level of each plot (Chapter 3). These lights are programmed to follow the natural photoperiod throughout the year and did not significantly affect air temperatures (De Frenne et al., 2015).

Addition, sampling and analysis of labelled $^{15}NH_4^+$ and $^{15}NO_3^-$

The uptake of mineral N was followed via four pulse additions of $^{15}NH_4{}^{15}NO_3$ (with ^{15}N at 98 atom % excess). A grid with 20 sections of 8.5 × 7 cm^2 each was placed over the mesocosms with each section receiving four injections (of 0.5 mL) in order to obtain a homogenous distribution of the ^{15}N tracer over the whole mesocosm .We thus applied 40 mL at each addition from a 187 mg N L^{-1} solution. The injection was performed with a syringe that could penetrate the soil at 1 cm depth. The total amount of ^{15}N tracer added, equalled 8 % of the average total soil N concentration or 29.9 mg N per mesocosm. We decided to add 8 % of the average total soil N concentration as tracer, to minimise a fertilisation effect but still ensuring sufficient recovery of ^{15}N. The injection was performed with a syringe that could penetrate the soil at 1 cm depth. The injections were repeated four times: once per week from the 19th of April until the 10th of May 2017. This is the time when community peak biomass develops with considerable species turnover in understorey communities (Jagodziński et al., 2016). We acknowledge that varying phenology between species can be the basis of differences in uptake of inorganic tracer N. Early-flowering species would have allocated N to leaves prior to the second half of April from either nutrient-storing organs (Klimešová et al., 2016), and inorganic or organic N from the soil (Miller, Bowman, & Suding, 2007). Later flowering species would only start with taking up N during this period. By applying the tracer in this time-window, we aim to infer the fate of the inorganic ^{15}N pulses when all species in the community are present and the community as a whole reaches peak biomass under multiple global change treatments.

We harvested at least two leaves per individual on 17th May 2017, and only leaves which emerged during that growing season. We pooled leaf samples of the same species within each mesocosm prior to ^{15}N analysis. *Glechoma hederacea* and *Geranium robertianum* were excluded from the analysis since an insufficient amount of leaf samples (<5) were collected. Leaf samples were dried at 65 °C for 48 h and milled for 5 min at 200 rpm (Mixer Mill MM 200, Retsch, Germany). Grass tissue samples were milled for 10 min to obtain the same fragmentation degree as the other plant species. Total leaf N content per leaf dry mass (%, gram gram^{-1} ×100) and ^{15}N abundance (at. %) were measured using an elemental analyzer (ANCA-SL, SerCon, UK) coupled to an isotope ratio mass spectrometer (20-22, SerCon, UK). The natural abundance of all species was, rounded on two digits, 0.37 % in the original planting material, so that this value is used throughout the analysis (Appendix Table A5.2.1). We expressed the enrichment in ^{15}N as a percentage of N derived from label (Ndfl%) (International Atomic Energy Agency, 1983), which gives the percentage of N in the leaves that are added via the tracer, with 0% being no tracer recovered (Equation 5.1).

$$Ndfl\% = \left(\frac{^{15}N_{leaf\ sample} - 0.37}{^{15}N_{excess\ NH_4NO_3}}\right) * \ 100$$

Equation 5.1. Calculation of the percentage N derived from label (referred to as "N derived from fertiliser" (Ndff) by International Atomic Energy Agency, 1983). The numerator consists of the measured ^{15}N abundance in a given leaf sample (at. %) subtracted with the natural abundance of ^{15}N in plant tissues (0.37 at. %). The denominator notes the abundance of ^{15}N in excess in the labelled NH_4NO_3, which was 98 at.%.

Measuring plant and community variables

We measured leaf N content per dry mass and specific leaf area (SLA) as the functional leaf traits in our analysis (for terminology, see Garnier et al. 2016). Leaf N content per dry mass (%) was measured during the analysis of ^{15}N isotopes and is described in the section above. We measured specific leaf area (SLA) from fresh leaves (excluding petioles) at the time of peak biomass (see Chapter 3 Table 3.1). We took digital RGB images at 30 cm height (13 MP camera) of the leaves on a white background with a red square reference area (4 cm^2). We calculated leaf area using the digital analysis tool *"Easy Leaf Area"* (Easlon & Bloom, 2014), which calibrates the area of green pixels in the image in relation to the red reference area. After measuring leaf area, samples were dried for 48 h at 65°C to ensure a constant dry weight. Specific leaf area was calculated as the ratio of the leaf area to dry mass and expressed in cm^2 mg^{-1}.

Total vegetation cover is a community property that is linked with biomass production, vegetation density, and asymmetric competition for light (DeMalach et al., 2017; Muukkonen et al., 2006). Total vegetation cover could thus influence N uptake, via a larger community biomass production and larger asymmetric competition for light, which could dilute leaf N. Total vegetation cover was measured three times during the experiment: before the first ^{15}N pulse (17th of April), at the third ^{15}N pulse (2nd of May) and just before the harvest of leaves for ^{15}N analysis (17th of May). We took digital RGB images at 75 cm height of the mesocosms perpendicular to the soil surface. We used the *Canopy Area* tool to measure green pixels of vegetation in these images and recalculate these values into a cover percentage (Easlon & Bloom, 2014). The vegetation cover ranged between 40% and 80% (see Figure 4.2B). These plant communities are consequently not overly dense, while individual leaves of plants can still overlap but remain recognisable The height of 75 cm is well above the average plant height in our experiment (see Chapter 4), so that unwanted side effects of overestimating tall species were limited. Individual species cover was later assessed manually by visually checking the digital images for the share of each species in the total vegetation cover of each mesocosm, because the "Canopy Area" tool cannot discriminate cover between species. We used this species cover value to weight the intraspecific SLA in each mesocosm in order to calculate a community weighted mean value of SLA (CWM SLA).

Data analysis

All hypothesis tests and data analyses were performed using R (R Core Team, 2019). We consistently applied linear mixed effects models (package *nlme,;* Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2018) with "Region" as a random intercept.

In hypothesis 1, we test direct and indirect effects of environmental changes on species' leaf N content per dry mass and Ndfl%. As this is a complex question, we used the package "piecewiseSEM" to build a structural equation model (SEM) (Lefcheck, 2016). The main idea of this type of SEM is to perform SEM analyses on hierarchically structured data. A certain response variable can be added as a predictor variable in a next model, with regression coefficients and p-values for each model calculated directly when fitting the SEM. To check whether the overall hypothesised relationships are consistent with the data, a Fischer C test is computed for which p has to be greater than 0.05. We test whether the environmental change affects community properties (CWM SLA and cover), which in turn affect leaf N and N tracer uptake within and between species. This order could arguably be opposite as well (i.e. putting species responses first). However, we believe that modelling the potential community cover increase in response to environmental change, which then affects leaf N, would be easier to understand these indirect effects. From the viewpoint of an indirect treatment effect, i.e. environmental change effects via changing community properties, it seems easier to model community change first. This community property change (i.e.vegetation cover and CWM SLA) can then affect individual species properties such as mean leaf N content. It would be more difficult to understand indirect effects via community properties if species are modelled first.

The hypothesised relationships allow checking for direct and indirect effects of the four environmental treatments (T, L, N, LU as main effects) on four partial responses. These are CWM SLA (a community trait characteristic, cm^2 leaf area mg^{-1} leaf dry mass), total vegetation cover (proxy for biomass production, %), leaf N content per dry mass (%) and the retrieved ^{15}N as Ndfl % (Figure 5.1A, Table 5.1). The first relationship shows the CWM SLA as a community response to the four environmental treatments, as we have assembled twelve communities with different species and leaf trait combinations. This relationship tests whether communities veer towards dominance of individuals and species with high SLA under bright, warm and nutrient enriched conditions, i.e. a resource acquisitive signature. The second is a response of total vegetation cover to the CWM SLA and the four environmental treatments. This relationship tests whether vegetation cover increases due to a changing community or to environmental treatments directly. The third is the response of leaf N content per dry mass (accounting for species identity) to the vegetation cover and the environmental treatments. This relationship tests whether enhanced community growth could cause a dilution of tissue N. We adopted leaf N content per dry mass in the SEM in favour of SLA because we expect that these two variables are highly correlated (see hypothesis 2) and that leaf N would potentially better reflect the uptake of N (see Firn et al. 2019).

The final relationship tests the ^{15}N retrieval (Ndfl%) to variation in species' leaf N content per dry mass (species identity effect + leaf N) together with direct effects of the four environmental treatments. In this model, two opposing trends in foliar N are tested: species can show interspecific differences in leaf N, but environmental changes can directly cause growth dilution of tissue N across species. We acknowledge that testing against the first species in the list (*A. podagraria)* could potentially bias our inference regarding interspecific differences in leaf N. However, we could assume a robust estimation of interspecific differences in leaf N between species (i.e. different intercept values), as this reference species has the "most average" leaf N among our species pool (Figure 5.2B). Prior to running the SEM, we tested these relationships for normality assumptions using a Shapiro-Wilk test and checking residuals with a histogram and qq-plot. We log-transformed the response variable if necessary to attain normality in residuals, which only applied to Ndfl%. We also checked the model plots of residuals vs fitted values to check for inconsistencies given the factors included in the model, which we did not observe. Residuals were independent of treatments (based on boxplots).

Table 5.1. Four separate linear mixed effects models feed into the piecewise structural equation model. The response of a former model becomes a predictor in the next, alongside the environmental treatments of land-use history (LU), warming (T), light addition (L) and Nitrogen enrichment (N). Two variables are measured on the community level: CWM SLA (community weighted mean of specific leaf area) and total vegetation cover (Cover). Leaf N content per dry mass (Leaf N) and the retrieval of ^{15}N (Nitrogen derived from label, Ndfl%) are measured within species, hence the addition of the species identity as a predictor.

Level	Response	Model
Community	CWM SLA	T + L + N + LU + 1|Region
Community	Cover	CWM SLA + T + L + N + LU + 1|Region
Species	leaf N	Identity + Cover + T + L + N + LU + 1|Region
Species	Log(Ndfl%)	Identity + leaf N + T + L + N + LU + 1|Region

After computing the SEM, we compared the effect sizes of direct vs indirect effects of experimental treatments on both leaf N content per dry mass and Ndfl%. Quantifying the indirect effect size of an environmental treatment is obtained by multiplying the coefficients of significant pathways that start from a given experimental treatment and indirectly lead to one of either focal response variables. We calculated net effects of each treatment by adding the significant direct and indirect effects, and computed a ratio of Direct:Indirect effects to compare effect sizes. The pathway of indirect effects for leaf N content per dry mass can potentially occur via CWM SLA and/or Cover, while the pathways for indirect effects of a treatment on Ndfl% can potentially occur via the net effect of that treatment on leaf N.

In hypothesis 2, we test whether the labelled ^{15}N will be retrieved most by fast-colonising nitrophilic species with high leaf N content per dry mass (%) and high specific leaf area (SLA, $cm^2 mg^{-1}$), i.e. resource acquisitive leaves (Díaz et al., 2016). We calculated Pearson's correlation coefficients (Pearson's r, alpha = 0.05) between the percentage N derived from label (Ndfl%) in leaves and leaf N content per dry mass, SLA and a species' colonisation capacity index (CCI). Estimates and confidence intervals were calculated with linear regression (in case of Ndfl% ~Species + CCI). To check for the relationships between the leaf traits and colonisation ability independently of Nfdl%, we computed Pearson's r between leaf N content per dry mass and SLA, leaf N content per dry mass and CCI, and SLA and CCI.

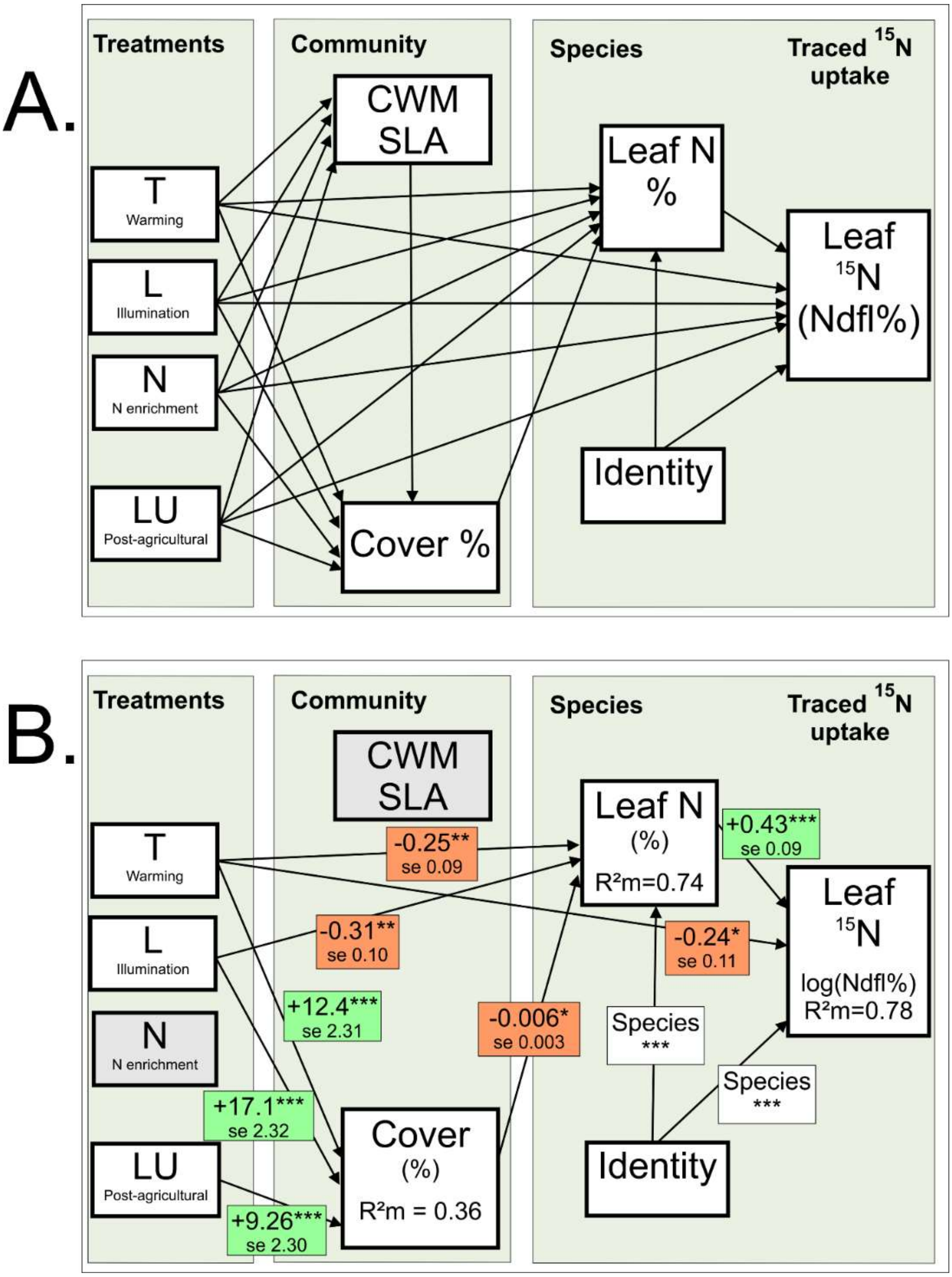

Figure 5.1. Structural equation model showing direct and indirect effects of environmental treatments on leaf N (%) and Ndfl%. Panel A. Graphical representation of the hypothesised structural equation. All four treatment variables are included to predict each of the four response variables: community weighted mean of SLA (CWM SLA, cm^2/mg), Total vegetation cover (Cover, %), leaf N content per dry mass (Leaf N %) and leaf N derived from label (Ndfl%). **Panel B.** The result of the analysis using piecewiseSEM, with the retained paths being in accordance with the data (Fischer C = 3.16, p =0.97). Only the significant ($p<0.05$) paths are shown here, for all coefficients see Appendix Table A5.3.1. The asterisks relate to the significance levels of the coefficients (*<0.05,**<0.01,***<0.0001). Marginal R^2 (fixed factors alone) are given here; the conditional R^2 (with random effects included) did not differ from these, meaning that no extra variation was explained by the random effect terms. The model estimates with standard errors are given in the units of the response variable per unit of change in the predictor variable for continuous variables. For categorical variables, these are relative to control conditions (for T, L) and relative to ancient forest (for LU). The estimates of the species- effects on leaf N and Ndfl% are given in Appendix Table A5.3.1. Treatment "N" and community variable "CWM SLA" are greyed out for not having significant effects on a response variable.

Results

In hypothesis 1, we tested direct and indirect effects of N enrichment, warming, enhanced light availability and agricultural land-use history on leaf N content per dry mass (%) and ^{15}N uptake (Ndfl%), mediated by the community vegetation cover (%) and community weighted mean of specific leaf area (CWM SLA) in a structural equation model (Figure 5.1A). The overall SEM had a Fischer C of 3.16 with a p-value of 0.98, which indicates that the retained relationships are a valid description of the system (for the full model description with all coefficients, see Appendix Table A5.3.1).

The N enrichment treatment and the CWM SLA did not significantly affect any response variable (Figure 5.1B). We found significant positive and direct main effects of past agricultural history (+9.26±2.3 S.E., $p<0.0001$, unit: %), enhanced light availability (+17.1±2.3 S.E., $p<0.0001$, unit: %) and warming (+12.4±2.3 S.E., $p<0.0001$, unit: %) on total vegetation cover (Figure 5.2A). The increasing total vegetation cover in response to environmental change in turn led to a lower species' leaf N content per dry mass (-0.006 ± 0.003 S.E., $p=0.03$, Figure 5.2B) which constitutes as an indirect effect. Direct negative effects of warming (-0.25±0.09 S.E., $p=0.0095$) and illumination (-0.31±0.10 S.E., $p=0.0013$) on leaf N content per dry mass across species occurred as well. Finally, the log-transformed ^{15}N uptake by species (log(Ndfl%)) had a direct negative response to warming (-0.23±0.11 S.E., $p=0.03$), but a positive response (+0.43±0.09 S.E., $p<0.0001$) to species' leaf N content per dry mass (%).

When comparing the effect sizes of the direct and indirect effects of the environmental treatments on both leaf N content per dry mass and Ndfl% (Table 5.2), we observed that significant direct effects were between 1.7 and 3.3 times larger than significant indirect effects of the same treatment. However, more significant indirect effects occurred for both leaf N content per dry mass (2 direct, 3 indirect) and Ndfl% (1 direct, 3 indirect). All significant direct and indirect effects of environmental treatments on both responses were negative, hence net effects of environmental treatments lowered both leaf N content per dry mass and Ndfl%. When summing significant effects of experimental treatments, we found that the direct effects of warming (T) and illumination (L) on leaf N (%) surpassed the indirect effects (from T,L,LU) via vegetation cover by 2.4 times. Notice that indirect effects on leaf N % occur via Cover, and on log(Ndfl%) via leaf N. For Ndfl%, the direct effect of warming (T) was 0.7 times smaller than the indirect effect of combined treatments via leaf N content per dry mass (originating from T,L,LU via vegetation cover). But leaf N% also directly affects Ndfl%, and the summed indirect effects of the treatments on log (Ndfl%) via leaf N (-0.341) did not cancel out the positive direct effect of leaf N % (+0.43, see Figure 5.1B).

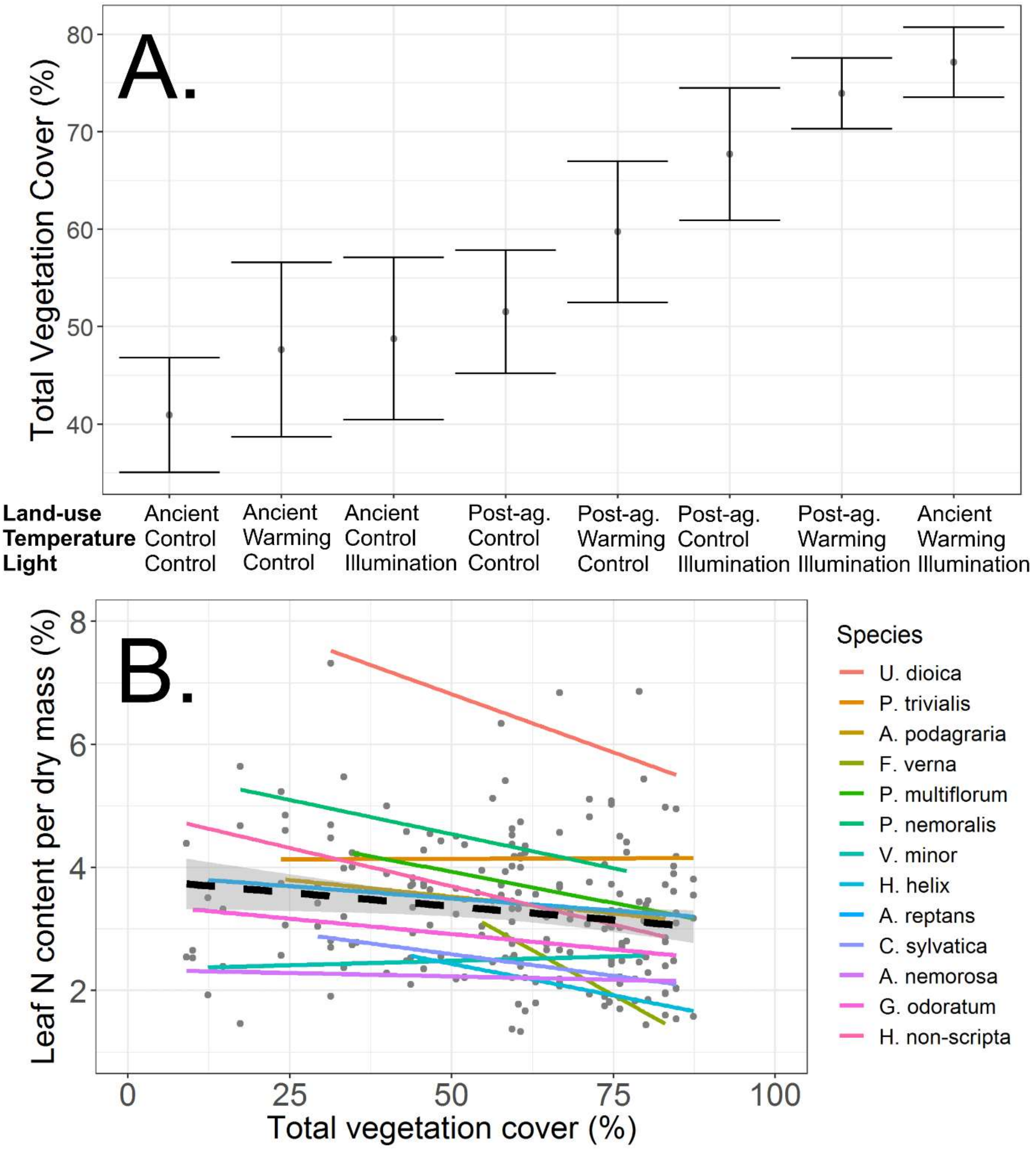

Figure 5.2. Environmental treatments increased community vegetation cover, which caused a dilution in leaf N content per dry mass (%) across species. Panel A: Significant positive main effects of past agricultural history (+9.26±2.30 S.E., p<0.0001), illumination (+17.1±2.32 S.E., p<0.0001) and warming (+12.4±2.31 S.E., p<0.0001) on total vegetation cover (%). The averages (95 % CI) within the replicates are shown here. **Panel B**: The bold dashed line (95% CI) represents the significant decrease (-0.006±0.003 S.E., p =0.03) of leaf N content per dry mass in response to total vegetation cover across species. The coloured lines gives the trend for each species. Species in the legend are ordered on colonisation capacity index (CCI), with the fastest colonisers on top. Notice that the species with that fits best with the average dashed line is *A. podograri*a, also the reference intercept value in the model analysis. We could thus assume a robust estimation of interspecific differences in leaf N between species, as this reference species has the “most average” leaf N among our species pool Averages for absolute values of leaf N per dry mass of each species can be found in Table A5.4.1.

Table 5.2. Partitioning of significant ($p<0.05$) direct and indirect effects of treatments warming (T), light addition (L), N addition (N) and agricultural land-use history (LU) on leaf N content per dry mass (leaf N %) (a) and leaf nitrogen derived from label (Ndfl%) (b). Coefficients for direct and indirect effects are obtained by multiplying the coefficients that were calculated by piecewise structural equation modelling (see Figure 5.1B) and are in unit of the response variable per treatment level. The net effect of a treatment is the sum of both the direct and indirect effect. When a treatment has both direct and indirect effects on a response variable, a ratio of Direct over Indirect effect is calculated for comparing the effect size. Insignificant effects are greyed out, see Table A5.3.1 for all model coefficients (including $p>0.05$). Notice that indirect effects on leaf N % occur via Cover, and on log(Ndfl%) via leaf N%. The summed indirect significant effects of the treatments on log (Ndfl%) via leaf N (-0.341) do not cancel out the positive direct effect of leaf N % (+0.43, see Figure 5.1B).

a)

Response	Treatment	Direct	Indirect (via Cover)	Net effect	Ratio Direct:Indirect
Leaf N (%)	T	-0.250	-0.074	-0.324	3.4
	L	-0.310	-0.103	-0.413	3.0
	N				
	LU		-0.056	-0.056	
	Sum	-0.560	-0.232	-0.792	2.4

b)

Response	Treatment	Direct	Indirect (via Leaf N)	Net effect	Ratio Direct:Indirect
log(Ndfl%)	T	-0.230	-0.139	-0.369	1.7
	L		-0.177	-0.177	
	N				
	LU		-0.024	-0.024	
	Sum	-0.23	-0.341	-0.571	0.7

In hypothesis 2, we tested whether ^{15}N retrieval in foliage is greatest in fast colonising nitrophilic species with resource acquisitive leaf traits. When looking at the correlations between the leaf traits and CCI, we found that leaf N content per dry mass and SLA were positively correlated (Pearson's $r = 0.54$, $p<0.0001$), and that leaf N content per dry mass (Pearson's $r =- 0.45$, $p<0.0001$) and SLA (Pearson's $r =-0.38$, $p<0.0001$) were both negatively correlated with CCI. More importantly, we found that the percentage N derived from label (Ndfl%) in leaves was negatively correlated with a larger affinity of plant species for ancient forest (Colonisation Capacity Index (CCI), Pearson's $r=-0.38$, $p<0.0001$, Figure 5.3A) and positively correlated with leaf N content per dry mass (unit: %, Pearson's $r=0.45$, $p<0.0001$, Figure 5.3B) and SLA (unit: cm^2 mg^{-1}, Pearson's $r = 0.64$, $p<0.0001$, Figure 5.3C). Looking at the three best colonisers (*Urtica dioica, Poa trivialis* and *Aegopodium podagraria*), these had an Ndfl% of over 6 %, with the highly competitive nitrophile *Urtica dioica* (Parzych, Jonczak, & Sobisz, 2017) even obtaining 9 % of its foliar N from the pulse additions. Contrastingly, the most N conservative species (Ndfl <1%) that we found were *Hyacinthoides non-scripta, Anemone nemorosa, Polygonatum multiflorum, Hedera helix* and *Vinca minor*, all understorey species typical of ancient forest.

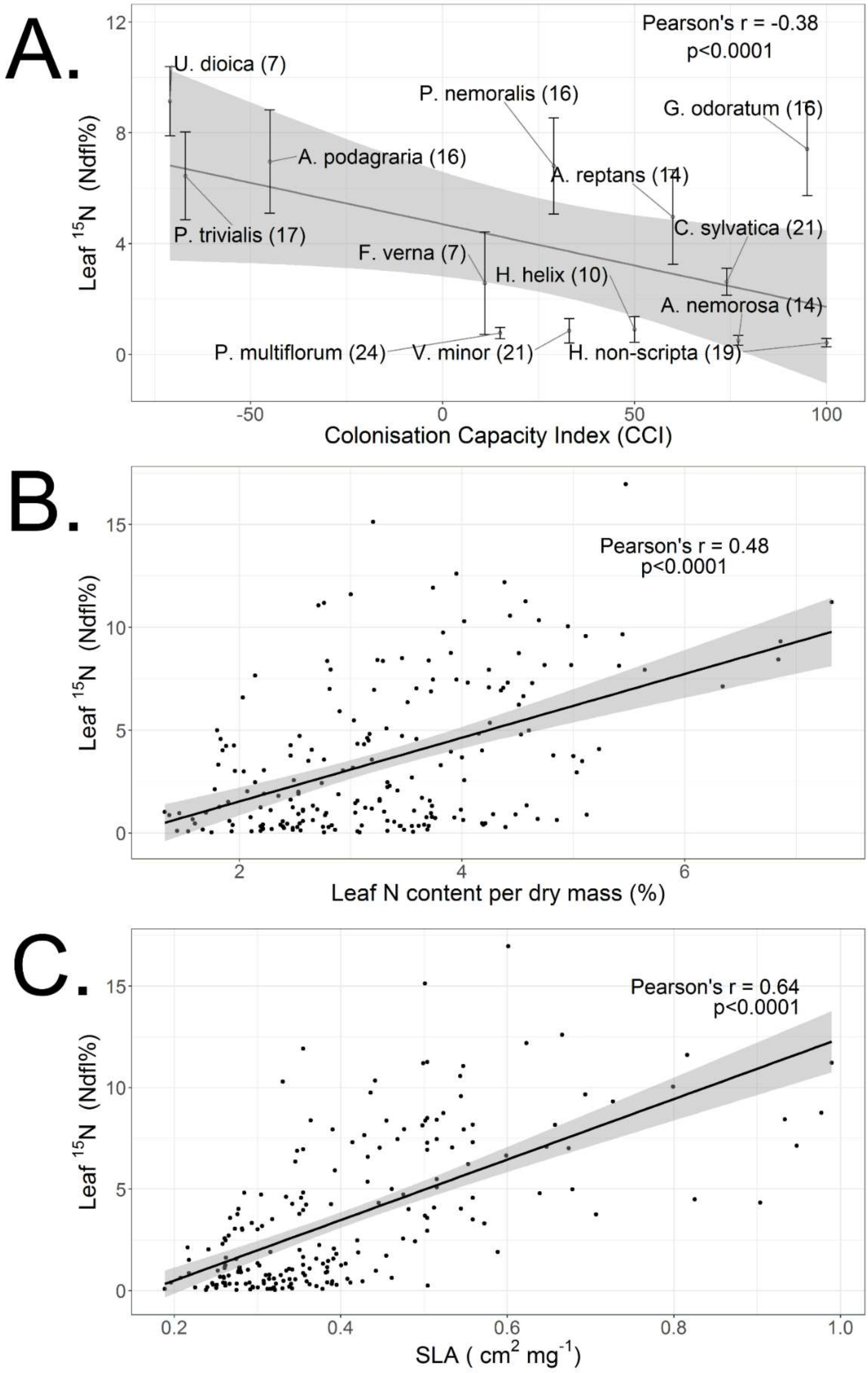

Figure 5.3. The labelled ^{15}N (Ndfl%) was retrieved most by resource acquisitive species with a fast colonisation into post-agricultural forest, a high leaf N content per dry mass (%) and high specific leaf area (SLA). Panel A. CCI correlated negatively (Pearson's r=-0.38, p<0.0001) with Ndfl% and is thus larger within fast colonisers. The number of leaf samples of each species are given alongside the species code. **Panel B.** Plants with a high leaf N content per dry mass had a higher Ndfl% (Pearson's r=0.48, p<0.0001). **Panel C.** SLA correlated positively with Ndfl% (Pearson's r = 0.64, p<0.0001). All confidence intervals are on the 95% confidence level.

Discussion

We documented plant N uptake of forest herbs under the influence of environmental change treatments and agricultural legacies. By measuring leaf N content per dry mass, we can infer responses of foliar N to environmental change independently of the N source or time of allocation. Applying a ^{15}N tracer at the height of spring allows us to infer the fate of inorganic N pulses in leaves at a time when all species in the community are present and the community as a whole reaches peak biomass under multiple global change treatments. The structural equation model confirmed our first hypothesis that environmental change treatments and agricultural legacies can have direct effects on leaf N content per dry mass and percentage leaf N derived from label (Ndfl%) with indirect effects via community properties. Of these treatments, warming and illumination were the most important, and their direct effects were between 1.7 and 3.4 times larger than their indirect effects. Additionally, we confirmed our second hypothesis that large interspecific differences in Ndfl% would correlate with a species' colonisation capacity and resource acquisition strategy. The results thus show that environmental changes directly and indirectly affect forest herb N uptake, and that the magnitude of this effect depends on the position of a species along the resource acquisitive – conservative continuum (Díaz et al., 2016; Reich, 2014; Wright et al., 2004).

Direct and indirect effects of environmental changes on plant N uptake

Using structural equation modelling, we were able to disentangle direct and indirect effects of agricultural land-use history and environmental changes on leaf N content per dry mass and the inorganic ^{15}N uptake in leaves (Ndfl%) within species. Contrary to our expectations, we found neither direct nor indirect effects of N enrichment and community weighted mean of SLA (CWM SLA) on any response variable. Furthermore, it is unlikely that the N enrichment treatment may produce interactions with other drivers to influence community responses (but see Dirnböck et al., 2017; Perring, Diekmann, et al., 2018; Schmitz et al., 2019), given the lack of such interactions in Chapters 4 and 6. We deliberately excluded interactions from the analysis as it would overcomplicate and convolute the SEM, and we mostly expected strong main effects. Due to the seasonal frequency of the N addition, main effects of N enrichment could also have been overshadowed by the rather high ambient N deposition in the test site (15.5 kg N ha^{-1}) which falls in the range of the critical load for deciduous forest (Bobbink et al., 2015). The lack of environmental treatments affecting CWM SLA suggests that there were no major shifts in community composition towards selection of species with high SLA under nutrient enriched, bright and warm conditions since the time of planting (2 years). The community cover did however increase in those conditions, but independently of CWM SLA.

Leaf N content per dry mass responded directly and indirectly to the treatments. We found direct negative effects of enhanced light availability and warming on leaf N content per dry mass.This effect could indicate that more foliar N is beneficial in low light environments within species, which could originate from a compensatory mechanism where plants invest more in foliar N under suboptimal conditions for photosynthesis (Niinemets, 1997). On the other hand, warm and bright conditions (canopy gaps) can increase photosynthetic rates (Paul-Limoges et al., 2017), which raises carbon acquisition and enhances plant growth, but with a higher tissue C:N ratio which reflects a tissue dilution of N (Jarrell & Beverly, 1981; Sardans et al., 2017; Takeuchi et al., 2001; Yin, 2002). This tissue dilution of N can have important effects for N cycling in the food web, as it is a mechanism that decreases the quality of leaf litter (Cornwell et al., 2008; Hobbie, 2015). We observed that the tissue dilution of N could be exacerbated by enhanced community growth via an indirect effect of environmental treatments. Warming, illumination and agricultural history together nearly doubled vegetation cover, which is a proxy for community biomass production (Heinrichs, Bernhardt-Römermann, & Schmidt, 2010; Muukkonen et al., 2006). This increase in vegetation cover explained additional variation of decreasing leaf N content per dry mass across species. This additional dilution effect might originate from asymmetric competition for light between individuals, which pushes plants to invest more in vertical growth (DeMalach et al., 2017, 2016; Freckleton & Watkinson, 2001). The effect size of this indirect effect via vegetation cover is, however, three times smaller than the direct lowering effects of illumination and warming on leaf N content per dry mass. In addition, the slope of this vegetation cover effect is subtle compared to the large interspecific differences in leaf N content per dry mass that we observed.

The percentage leaf N derived from label (Ndfl%) responded to the leaf N content per dry mass of species and the warming treatment. The direct positive effect of leaf N content per dry mass showed that there are large interspecific differences in Ndfl%, and these differences can be predicted by a species' average leaf N content (%). This positive direct effect on Ndfl% did not cancel out when accounting for the indirect negative effects of combined environmental treatments (T, L, LU) on leaf N content per dry mass. In addition, warming was the only environmental treatment that directly affected Ndfl%, and reflected a lower Ndfl% in warmer conditions. A similar negative effect was found in leaf N content per dry mass, and may also result from a tissue dilution in N due to enhanced growth (Jarrell & Beverly, 1981). Overall, we found that species' innate leaf N content remains the main predictor for foliar N uptake (as measured by Ndfl%) as its effect size is nearly double than that of the warming treatment.

N uptake by forest herbs follows the fast – slow growth continuum of plant forms

At the time of community peak biomass and across environmental treatments, fast colonising forest herbs with acquisitive leaf traits had more leaf N derived from label (Ndfl%) than slow colonising and resource conservative species. These results are intuitive and expected (Tilman, 1990), but this is to our knowledge the first time where this has been tested using a N tracing experiment in temperate forest herbs and across multiple environmental manipulations (for other ecosystems, see Andersen and Turner 2013; de Vries and Bardgett 2016). Our N tracing experiment confirms that forest herbs follow the global fast – slow continuum of functional plant traits and strategies (Reich, 2014). This fast-slow continuum of plant forms arises from resource acquisition and leaf construction (Díaz et al., 2016; Wright et al., 2004), its connection with fast and slow life-history strategies (Adler et al., 2014; Reich, 2014) and dispersal ability (Beckman et al., 2018). We found that species that naturally grow in post-agricultural forests retrieved more of the inorganic tracer N in their leaves. These species, e.g. *Urtica dioica,* have higher relative growth rates and accumulate biomass until the end of the growing season (Fichtner & Schulze, 1992), unlike spring flowering (vernal) species who quickly stall in growth once they reach peak biomass (Paul-Limoges et al., 2017).

Caution in inference of the experimental findings

We identified four potential limitations to our study, which need to be addressed to properly infer the findings from our experimental study. First, the phenology and stalled growth of vernal species towards May likely contributed to a lower Ndfl% in their leaves in comparison with the fast colonisers, which tend to flower later in the growing season. However, our finding of a lower leaf N content per dry mass in slow colonisers still suggests that these early flowering species are N conservatives (as in Hofmeister et al. 2012). Second, species that had already reached peak aboveground biomass before our tracing period (half of April) would have built up N from other sources (including organic and inorganic soil N) and could have allocated the tracer N to roots or other nutrient storing organs (Miller et al., 2007). We did not sample any below ground traits to avoid damage to the communities, and instead were interested in responses of foliar N to environmental change treatments. Such allocation patterns of N to roots in favour of leaves can however differ under environmental changes, depending on plant functional types (Freschet, Swart, & Cornelissen, 2015; Sardans et al., 2017; Sugiura & Tateno, 2011) and the preferred N source of species such as organic N (Miller et al. 2007). Whether organic N uptake could alter in forest herbs due to environmental change is speculative but worthy of further research.

A third issue is a potential inhibiting effect of labile Al on the uptake of inorganic N in plants, which could vary due to the forest land-use history (Burnham, Cumming, Adams, & Peterjohn, 2017). While labile Al can inhibit N uptake in many species, it is likely that the forest herbs of our species pool are less affected by the adverse effects of Al. Many woodland species prefer to utilise NH_4^+, the more common inorganic N form in slightly (pH < 5) acidic forest soils (Falkengren-Grerup, 1995). Instead, soluble Al can strongly decrease the availability of NO_3^- indirectly, via an inhibition on nitrifying microbes (Yang, Fan, & Ryan, 2018), which is of less importance in our experimental setup because we added inorganic NH_4NO_3 to our mesocosms directly. Furthermore, the availability in labile soil Al varied more strongly between regions than it did between pairs (i.e. due to land-use history, see Table A5.1.1), so that any variation in Al is largely taken into account with the hierarchical modelling.

A final issue is the possibility that a limited N supply (N limitation) was a driver for dilution in tissue N, rather than changing N allocation patterns in response to environmental change. In a supplementary analysis (Appendix 5.4), we compared the maximal N demand of aboveground biomass in the mesocosms to a conservative estimate of the N supply (from available inorganic soil N pool and N deposition) to check whether an N deficit could occur. If N demand calculated at maximum possible observed leaf N content per dry mass is always lower than the minimum N supply, then N limitation is an unlikely explanation for any altered N allocation to the leaves under changing environmental conditions. To be unequivocal in ruling out N limitation, it would be necessary to also include the N demand for belowground biomass (i.e. roots), but also the N that is supplied via mineralisation of organic matter. We chose not to perform any destructive measures to our plant communities, so that we instead approach these estimates conservatively by accounting for aboveground biomass as N demand, and only N deposition and the available inorganic soil N as N supply. Our estimates show that maximal N demand from aboveground biomass was lower than the minimal supply in all instances (in most cases, substantially lower, see Table A5.4.1 and Figure A5.4.1). This means that N limitation is not a likely driver for changing leaf N. Furthermore, we found that current estimates of N stocks in aboveground biomass still increase due to larger productivity under environmental change despite a dilution of tissue N (Table A5.4.3, Table A5.4.4). Given that N stocks in aboveground biomass increased strongly with larger total vegetation cover, and no mesocosms surpassed their N supply, our results imply that N is a non-limiting resource. A lack of N limitation may be further demonstrated by the absence of an effect of the N fertilization treatment in our model analyses (Figure 5.1B, Table A5.4.3).

Forest herbs and N uptake: implications for conservation in a changing world

The results of our N tracing experiment could explain the poor representation of typical forest herbs in post agricultural forests, beyond the usual explanation of poor dispersal and subsequent establishment. We observed a differentiated inorganic N uptake between slow and fast colonisers during the growing season's peak of community turnover. This differentiated N uptake could exemplify common hurdles for biological conservation in ancient forest (Foster et al., 2003) and has potential effects for ecological restoration of post-agricultural forest (Honnay et al., 2002). The most N conservative species (Ndfl <1%) that we found were *Hyacinthoides non-scripta, Anemone nemorosa, Polygonatum multiflorum, Hedera helix* and *Vinca minor*, all understorey plants of conservation concern in Western Europe (Hermy, Honnay, Firbank, Grashof-Bokdam, & Lawesson, 1999). Threats to these flora in ancient forest are well documented (Baeten, De Frenne, Verheyen, Graae, & Hermy, 2010; Gilliam, 2007), as communities shift towards higher abundance of competitive and resource acquisitive species in response to global changes (Bernhardt-Römermann et al., 2011; Gilliam et al., 2016).

Contrastingly, the fate of slow colonising and resource conservative species in post-agricultural forests is less well-known as they naturally hardly occur there (Honnay et al., 2002). Development of forest understoreys can take decades up to centuries if abiotic conditions are suitable again for these species (De Frenne, Baeten, et al., 2011; Holmes & Matlack, 2018). Individual slow colonising species can, however, gradually increase (in abundance over time towards a community assembly akin to that of ancient forest Kolk et al., 2016; Naaf & Kolk, 2015). However, the gradual assembly of slow colonising species into post-agricultural forest could create novel species interactions with the resident fast N species (Alexander et al., 2015) and lead to a potential competitive exclusion of slow colonisers in the long run (Baeten & Verheyen, 2017). Successful biological introductions in these forests could therefore be difficult to accomplish if light and nutrient availability is high, with global environmental change inducing an additional level of uncertainty. Forest herbs differing in N uptake, and potentially competitive performance, may further compromise the ability of these species to establish and recruitment in post-agricultural forest. Reintroduction experiments of a broad range of typical forest species in post-agricultural forest would enable testing of these contentions.

Conclusions

Our work assessed forest herb performance under environmental change from a distinct mechanistic angle by measuring *in situ* plant N uptake in leaves. We found that environmental changes directly altered allocation of N to leaves in plants, with simultaneous but smaller indirect effects by affecting community properties that influence individual plant responses. We demonstrated that spectra in resource acquisition strategies (Adler et al., 2014; Beckman et al., 2018; Díaz et al., 2016; Reich, 2014; Wright et al., 2004) can be used to predict such plant responses to multiple environmental changes. Future directions which focus on the role of forest herb communities in the cycling of N from multiple sources would be worthy of further research. The insights that we have gained are relevant for conservation and restoration of herbaceous communities in temperate forest.

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Appendix 5.1. Attributes of selected mesocosms

Table A5.1.1. Summary of soil attributes of the samples. The sample code consists of the regional origin, and is either from regions Flanders, Belgium (“VL”) and Skane, Sweden (“SW”). The “A” stands for Ancient forest (in existence before 1850) and “R” stands for recent post-agricultural forest (established c. 1950).The number denotes the pair, where two closely located forest patches make up one pair. Legacies of prior agriculture are most evident in Olsen P and total P concentration, C:N and pH (see Blondeel et al. 2019 for further details and extraction processes and Chapter 3). For quantifying available N in NH4+ and NO3- forms (ISO/TS 14256-1), we measured a subsample of 3 g sieved fresh soil that was extracted with 10 ml 1 M KCl, shaken for 1 h (150 rpm), and filtered prior to analysis of NH4+ and NO3– concentrations. Ammonium was determined colorimetrically by the salicylate-nitroprusside method on an auto-analyzer (AA3, Bran Luebbe, Germany). Nitrate was determined colorimetrically using the same auto-analyzer after reduction of NO3– to nitrite (NO2–) in a Cu-Cd column, followed by the reaction of NO2– with N-1- napthylethylenediamine to produce a chromophore. The inorganic N stock is calculated from the sum of NO3-N and NH4-N, multiplied by the volume of soil sample (13 L), the average soil bulk density (1050 g/L), and divided by the surface area of the mesocosms (0. 315 x 0.465 m^2). The Aluminium content is from the Ammonium Lactate extraction, which indicates the potential labile Al pool.

Sample	Long.	Lat.	P_{olsen} (mg/kg)	$P_{\mathbf{total}}$ (mg/kg)	Ca (mg/kg)	Fe (mg/kg)	C_{inorg} (%)	C_{org} (%)	N_{tot} (%)	C_{org}:N_{tot} (mg/kg)	NO_3-N (mg/kg)	NH_4-N (mg/kg)	Inorganic N stock (g m^{-2})	pH	Clay (%)	Silt (%)	Sand (%)	BS	Al (mg/kg)
VL-A1	3.80259	50.97501	23	276	1015	10628	0.1	4.3	0.33	12.9	16.6	21.5	3.55	4.2	15	46	39	0.65	326
VL-R1	3.8024	50.97536	93	752	1339	16999	0.06	2.1	0.21	9.9	7.2	21.8	2.70	4.5	23	57	20	0.67	379
VL-A3	4.57108	51.19906	17	331	1549	8874	0.08	3.7	0.32	11.4	5.2	27.5	3.05	4.8	12	36	52	0.76	300
VL-R3	4.57092	51.19965	29	544	2507	9323	0.1	2.9	0.28	10.3	5.3	8.4	1.28	5.5	16	37	47	0.86	251
SW-A1	13.20699	55.55432	25	438	1224	11244	0.04	4.8	0.36	13.5	6.4	45.7	4.86	4.0	13	21	66	0.43	528
SW-R1	13.20736	55.5517	49	630	1925	11158	0.04	1.9	0.17	11.1	6.7	15.7	2.09	5.1	5	32	64	0.64	379
SW-A3	13.30391	55.5577	9	315	1397	8483	0.02	1.9	0.16	11.9	16.4	8.8	2.35	4.7	19	23	59	0.36	346
SW-R3	13.33121	55.54688	81	986	3599	16159	0.03	2.4	0.22	10.9	8.8	25.1	3.16	5.4	32	30	38	0.81	347

Table A5.1.2. Summary of the selected 64 mesocosms, with the communities listed for each environmental treatment (8 combinations) and soil sample (8). The two-level factorial combinations of the Warming, Light and Nitrogen enrichment treatments are given in each column, where the Control is the situation without any treatment.

Sample	**Control**	**Warming**	**Light**	**Nitrogen**	**Warming + Light**	**Warming + Nitrogen**	**Light + Nitrogen**	**Warming + Light + Nitrogen**
VL-A1	com3	com1	com11	com2	com10	com12	com5	com4
VL-R1	com8	com12	com4	com3	com2	com10	com1	com5
VL-A3	com8	com1	com3	com5	com6	com11	com2	com4
VL-R3	com10	com8	com4	com7	com9	com11	com2	com6
SW-A1	com6	com11	com12	com9	com4	com8	com10	com1
SW-R1	com5	com4	com12	com10	com1	com9	com3	com2
SW-A3	com2	com9	com3	com6	com11	com7	com5	com10
SW-R3	com4	com3	com12	com10	com8	com6	com7	com9

Appendix 5.2. Natural abundance of ^{15}N in each species

Table A5.2.1. The natural abundance of ^{15}N (atm%) from a control population (four pooled leaf samples of original plant material grown in uniform substrate). There was no variation in natural abundance in ^{15}N (atm%) when rounded upon two digits, so that this value was used throughout the analysis.

Species name	**Mean ^{15}N (atm%)**	**Standard deviation**	**Rounded mean value**
Urtica dioica	0.36694	0.00022	0.37
Poa trivialis	0.36762	0.00045	0.37
Aegopodium podagraria	0.36654	0.00033	0.37
Geranium robertanium	0.36721	0.00045	0.37
Glechoma hederacea	0.36582	0.00035	0.37
Ficaria verna	0.36602	0.00029	0.37
Polygonatum multiflorum	0.36813	0.00104	0.37
Poa nemoralis	0.36872	0.00034	0.37
Vinca minor	0.36628	0.00033	0.37
Hedera helix	0.36585	0.001	0.37
Ajuga reptans	0.36704	0.00044	0.37
Carex sylvatica	0.3667	0.00054	0.37
Anemone nemorosa	0.36656	0.00058	0.37
Galium odoratum	0.36656	0.00027	0.37
Hyacinthoides non-scripta	0.36739	0.00022	0.37

Appendix 5.3. Additional statistical analysis

Table A5.3.1. Model estimates and standard errors (S.E.) from piecewise structural equation modelling for the four response variables. Effects of species are relative to the first species in the list (in alphabetical order) which is “Aegopodium podagraria” (abbreviated as Aegpod).

Response	Predictor	Estimate	S.E.	P-value	Threshold
CWM SLA(mm^{2}mg)	T	-0.02	0.01	0.098	
	L	-0.02	0.01	0.110	
	N	0.00	0.01	0.982	
	LU	0.00	0.01	0.901	
Cover (%)	T	12.35	2.32	0.000	***
	L	17.13	2.32	0.000	***
	N	-0.48	2.31	0.834	
	LU	9.26	2.30	0.000	***
	CWM SLA	-13.26	11.72	0.259	
Leaf N (%)	T	-0.25	0.09	0.010	**
	L	-0.31	0.10	0.001	**
	N	0.16	0.09	0.063	
	LU	-0.12	0.09	0.190	
	Cover	-0.01	0.00	0.031	*
	Ajurep	0.16	0.22	0.483	
	Anenem	-1.24	0.22	0.000	***
	Carsyl	-0.95	0.20	0.000	***
	Ficvern	-0.91	0.27	0.001	**
	Galodo	-0.47	0.21	0.030	*
	Hedhel	-1.24	0.24	0.000	***
	Hyanon	0.26	0.20	0.199	
	Poanem	1.09	0.21	0.000	***
	Poatriv	0.88	0.21	0.000	***
	Polmul	0.30	0.19	0.121	
	Urtdio	2.85	0.27	0.000	***
	Vinmin	-0.89	0.20	0.000	***
log(Ndfl%)	T	-0.24	0.11	0.033	*
	L	-0.10	0.11	0.369	
	N	-0.11	0.11	0.283	
	LU	-0.04	0.10	0.716	
	leafN	0.43	0.09	0.000	***
	Ajurep	-0.20	0.27	0.448	
	Anenem	-2.12	0.28	0.000	***
	Carsyl	-0.38	0.25	0.135	
	Ficvern	-0.81	0.34	0.017	*
	Galodo	0.44	0.26	0.092	
	Hedhel	-1.47	0.31	0.000	***
	Hyanon	-3.08	0.24	0.000	***
	Poanem	-0.31	0.27	0.254	
	Poatriv	-0.22	0.26	0.411	
	Polmul	-2.28	0.23	0.000	***
	Urtdio	-0.64	0.41	0.121	
	Vinmin	-2.14	0.25	0.000	***

Appendix 5.4. Ruling out N limitation as a driver for changing leaf N

Introduction

N limitation for plant growth is the phenomenon when N supply in a system does not meet plant demand for N, which limits plant growth and biomass production. N limitation can consequently induce changes in tissue N content of individual plants as plants adjust to the low N availability in the system. This mechanism of N limitation could confound our findings of the environmental change effects on leaf N and N uptake that we have presented in our work.

In this supplementary section, we check whether N limitation could be a driver for changes in leaf N and uptake of tracer N, by comparing N supply to N demand. We expect that N limitation does not occur, given the limited growth responses of the forests herb communities to the N addition treatment (see Figure 5.1B and Table A5.3.1). The lack of an N deficit would further rule out N limitation as a driver for changing leaf N. We thus expect that any changes in leaf N and N uptake could be attributed to environmental change treatments directly or indirectly (via changing plant community properties). We specifically test whether:

1. N supply in mesocosms could meet maximal N demand of the aboveground biomass, thus casting doubt on N limitation for production of aboveground biomass.
2. N stocks in aboveground biomass could still increase due to increased productivity in bright, warm and nutrient enriched conditions (agricultural legacies and N addition), despite the dilution effects that these environmental treatments have caused on leaf N content and N uptake.

Methods

We checked whether changing N stocks in aboveground biomass could reveal that N limitation is a driver for any changes in N allocation to leaves. We therefore estimated the N demand by the aboveground biomass in the mesocosms, and compared this to a conservative estimate for N supply (from inorganic soil N, N deposition, and when relevant the N treatment). To be unequivocal in ruling out N limitation, it would be necessary to also include the N demand for belowground biomass (i.e. roots), but also the N that is supplied via mineralisation of organic matter. Due to limitations in data collection (i.e. not wanting to take destructive measurements), we approach these estimations conservatively by accounting for aboveground biomass as N demand, and only N deposition and the available inorganic soil N as N supply.

To estimate the maximal N demand for aboveground biomass for each mesocosm, we calculated the N stock (g N m^{-2}) in aboveground biomass while species maintain their maximal leaf N content per leaf dry mass. To estimate community biomass, we used the phytocalc model (Heinrichs et al., 2010), which estimates species' aboveground biomass (unit: g N m^{-2}) from the shoot length of species, its cover, and three parameters that are specific to a species' life form. We measured species' shoot lengths in all mesocosms at the time of collecting leaves (17th May). We then multiplied these biomass estimates by species' highest recorded leaf N content per dry mass values to calculate a maximal N demand in aboveground biomass (Table A5.4.1). A current N demand of aboveground biomass (N stock) was calculated based on the leaf N content per dry mass of each species unique to a mesocosm.

We then graphically compared the N demand across the range of vegetation covers found in the experiment with an initial, conservative level of N supply. This N supply (4.43 g N m^{-2}) was calculated as the sum of inorganic N deposition (1.55 g N m^{-2}) and the average inorganic soil N concentration (NO_3-N and NH_4-N, see Table A5.1.1) from across all control mesocosms (2.88 g N m^{-2}). In case of the N addition treatment, mesocosms received an additional 5 g N m^{-2}. If N demand calculated at maximum possible observed leaf N content per dry mass is always lower than the minimum N supply, then N limitation is an unlikely explanation for any altered N allocation to the leaves under changing environmental conditions. If estimated aboveground demand is substantially lower than estimated belowground soil supply, then N limitation can virtually be ruled out, although demand would need to take into account belowground biomass for this to be unequivocal. A lack of N limitation may be further demonstrated by the absence of an effect of the N fertilization treatment in our model analyses.

Table A5.4.1. Maximal value of leaf N content per dry mass ("leaf N concentration", %) of each species in the experiment.

Species	Mean leaf N per dry mass (% = g g^{-1}x100)	Standard deviation leaf N per dry mass (% = g g^{-1}x100)	Highest observed leaf N per dry mass (% = g g^{-1} x 100)
Aegopodium podagraria	3.50	0.65	4.69
Ajuga reptans	3.37	0.85	4.82
Anemone nemorosa	2.24	0.37	2.7
Carex sylvatica	2.40	0.38	3.06
Galium odoratum	2.85	0.88	5.11
Hedera helix	2.09	0.71	3.35
Hyacinthoides non-scripta	3.73	0.66	4.85
Poa nemoralis	4.49	0.73	5.64
Poa trivialis	4.14	0.65	5.41
Polygonatum multiflorum	3.62	0.55	5.12
Ficaria verna	2.55	0.62	3.46
Urtica dioica	6.10	0.97	7.32
Vinca minor	2.51	0.56	3.55

In the second analysis, we tested whether N stocks in aboveground biomass would still increase with increasing total vegetation cover due to treatments of light addition, warming, agricultural land-use history, despite their observed dilution effects in tissue N. Therefore, we calculated the net effect of these environmental treatments, and that of N addition, on current N demand in aboveground biomass (N stocks) via a structural equation model (SEM, see Table A5.4.2). This structural equation model is a simplified version of the original model (Figure 5.1A in main manuscript), but with the pathways of CWM SLA omitted due the lack of its importance as a response or predictor variable. In this SEM, the first partial model accounts for Cover in response to the treatments (T, L, N, LU). The second model accounts for leaf N in response to species identity, Cover and the treatments (T, L, N, LU). The final model with community N stock as a response has Cover, leaf N and the treatments (T, L, N, LU) as predictors.

Results and Discussion

Maximal N demand from aboveground biomass was lower than the minimal N supply in all instances (Figure A5.4.1). Only two mesocosms managed to surpass the lowest N supply threshold of 4.43 g N m^{-2}, but these mesocosms received the N addition, so that minimal N supply is raised by 5 g N m^{-2}. Nitrogen is thus not likely to be a limiting nutrient in any mesocosm. This could only be unequivocally confirmed by assessing total plant N demand i.e. including N in belowground biomass.

In the second analysis, we tested whether current N stocks in aboveground biomass increase with larger vegetation cover, despite dilution effects of tissue N due to environmental treatments. N stocks in vegetation increased strongly when total vegetation cover increased (Table A5.4.3), i.e. when more biomass is produced (adding 1.6 g N m^{-2} when all treatments are applied). The only significant direct effect that we found was a positive effect from the N enrichment treatment on aboveground N stock (+0.27g N m^{-2}). All net effects of treatments were positive and together nearly doubled N stocks in aboveground biomass (+1.23 g m^{-2}, Table A5.4.4). In comparison, the decrease in N stock due to dilution of leaf N in species is small (-0.07 g N m^{-2}). These results thus confirm that N stocks still increase under environmental change despite a dilution of tissue N.

Given that N stocks in aboveground biomass increased strongly with larger total vegetation cover, and no mesocosms surpassed their N supply, our results imply that N is a non-limiting resource. Our initial explanation, that N limitation is not a likely driver for changing leaf N but that leaf N changes due to direct effects of temperature and light, is thus valid based on the comparison of the maximal N demand of aboveground biomass with a conservative estimate of N supply.

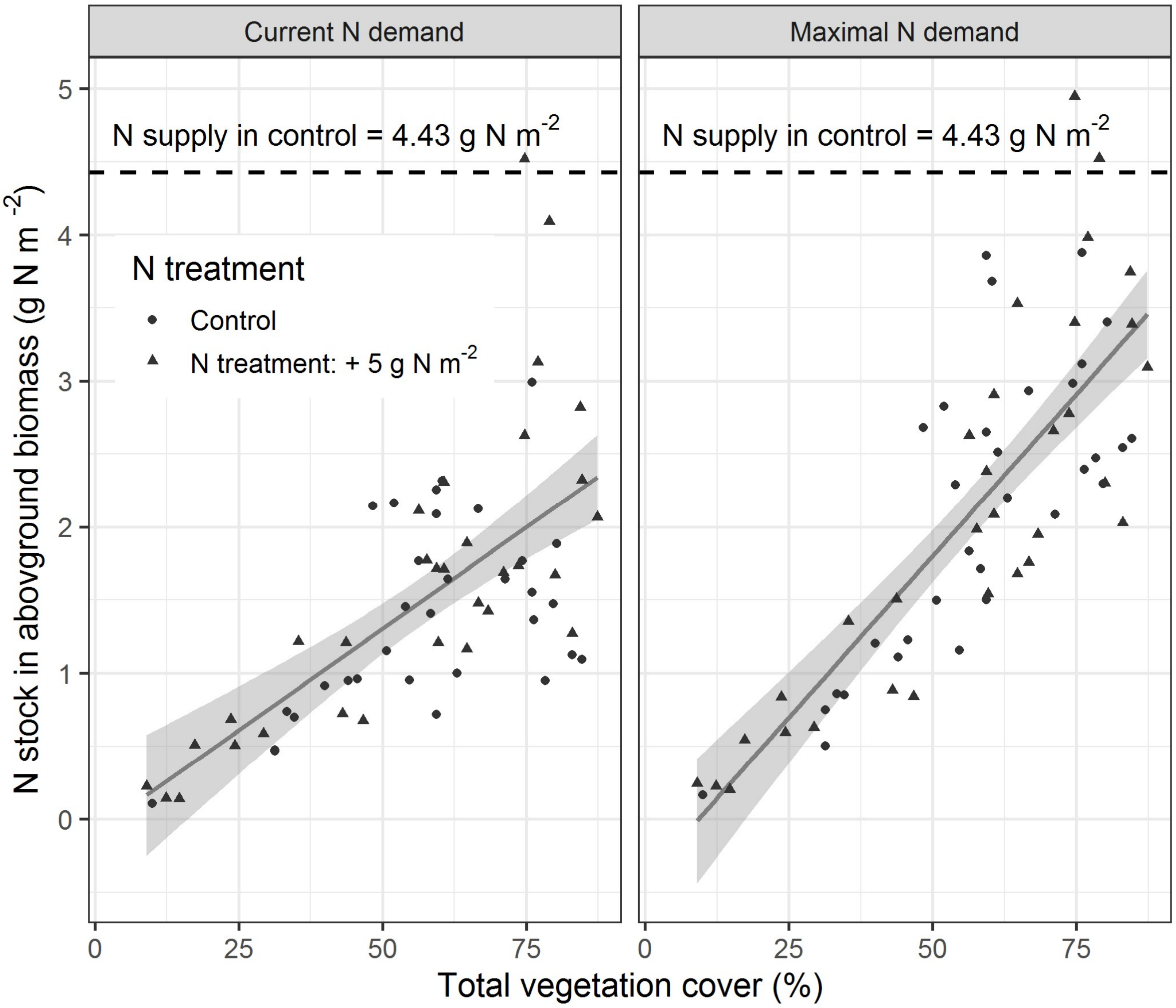

Figure A5.4.1. Current and maximal N demand of aboveground biomass for all mesocosms, across the range of observed total vegetation cover. These values do not account for N in belowground biomass. The current N demand (left panel) accounts for the actual leaf N content per dry mass of each species in the mesocosms, so that dilution effects in response to treatments and vegetation cover are taken into account. Contrastingly, the maximal N demand (right panel) does not account for declining leaf N responses and uses the highest recorded leaf N content per dry mass for each species (see Table A5.4.1) to calculate a standing N stock in aboveground biomass. The N supply threshold of controls (4.43 g N m-2) was estimated by calculating the average inorganic N stock in the mesocosm soils (2.88 g N m^{-2}, see) Table A5.1.1) and adding this with the N deposition in the site (1.55 g N m-2). Mesocosms in the N addition treatment (triangular points in figure) receive an additional 5 g N m-2 due to fertilisation with NH4NO3 (50 kg N ha1 eq.).

Table A4.5.2. Piecewise structural equation model for identifying changes in aboveground N stocks. Four separate linear mixed effects models feed into the piecewise structural equation model, where the response of a former model becomes a predictor in the next, alongside the environmental treatments of land-use history (LU), warming (T), light addition (L) and Nitrogen enrichment (N). Leaf N content per dry mass (Leaf N) is measured within species, hence the addition of the species identity (ID) as a predictor. The final response is the community N stock, which is determined by leaf N, total vegetation cover, and the environmental treatments.

Level	Response	Model
Community	Cover	T + L + N + LU + 1|Region
Species	leaf N	Identity + Cover + T + L + N + LU + 1|Region
Community	N stock	Cover + leaf N + T + L + N + LU + 1|Region

Table A5.4.3. Model estimates and standard errors (SE) from piecewise structural equation modelling for modelling change in community N stocks. Effects of species are relative to the first species in the list (in alphabetical order) which is "Aegopodium podagraria" (abbreviated as Aegpod).

response	predictor	estimate	SE	P value	Threshold
Cover	T	12.662	2.306	0	***
	L	17.430	2.303	0	***
	N	-0.488	2.307	0.8328	
	LU	9.240	2.306	1.00E-04	***
leaf N	T	-0.249	0.095	0.0095	**
	L	-0.314	0.096	0.0013	**
	N	0.161	0.086	0.0634	
	LU	-0.116	0.088	0.1904	
	Cover	-0.006	0.003	0.0313	*
	Ajurep	0.156	0.222	0.483	
	Anenem	-1.239	0.219	0	***
	Carsyl	-0.955	0.197	0	***
	Galodo	-0.465	0.213	0.0301	*
	Hedhel	-1.236	0.243	0	***
	Hyanon	0.261	0.202	0.1989	
	Poanem	1.087	0.211	0	***
	Poatriv	0.884	0.210	0	***
	Polmul	0.301	0.193	0.1207	
	Ranfic	-0.911	0.274	0.0011	**
	Urtdio	2.846	0.271	0	***
	Vinmin	-0.892	0.199	0	***
N stock in aboveground biomass	T	0.096	0.098	0.3268	
	L	-0.145	0.103	0.1607	
	N	0.269	0.091	0.0034	**
	LU	-0.083	0.094	0.3778	
	Cover	0.030	0.003	0	***
	leaf N	0.107	0.042	0.0117	*

Table A5.4.4. Direct and indirect effect of environmental treatments of land-use history (LU), warming (T), light addition (L) and Nitrogen enrichment (N) on N stocks in aboveground biomass (g N m^{-2}). The net effect is the sum of the direct and indirect effects of the treatments. All treatments had a positive net effect, which was most strongly mediated by an increased productivity.

Treatment	Direct	Indirect (via Cover)	Indirect (via leaf N)	Net effect
T	0.096	0.380	-0.035	0.441
L	-0.145	0.523	-0.044	0.334
N	0.269	-0.015	0.017	0.271
LU	-0.083	0.277	-0.006	0.188
Sum	0.137	1.165	-0.069	1.233

Image: Sofie Verstraelen

Chapter 6

Light and warming drive forest understorey community development in different environments

Adapted from: Haben Blondeel, Michael P. Perring, Leen Depauw, Dries Landuyt, Pieter De Frenne, Kris Verheyen. *Light and warming drive forest understorey community development in different environments*. Global Change Biology (resubmitted after major revisions).

Abstract

Plant community composition and functional traits respond to chronic drivers such as climate change and nitrogen (N) deposition. In contrast, pulse disturbances from ecosystem management can additionally change resources and conditions. Community responses to combined environmental changes may further depend on land-use legacies. Disentangling the relative importance of these global change drivers is necessary to improve predictions of future plant communities. We performed a multi-factor global change experiment to disentangle drivers of herbaceous plant community trajectories in a temperate deciduous forest. Communities of five species, assembled from a pool of fifteen forest herb species with varying ecological strategies, were grown in 384 mesocosms on soils from ancient forest (forested since at least 1850) and post-agricultural forest (forested since 1950) collected across Europe. Mesocosms were exposed to two-level full-factorial treatments of warming, light addition (representing changing forest management) and N enrichment. We measured plant height, specific leaf area (SLA), and species cover over the course of three growing seasons. Increasing light availability followed by warming reordered species towards a taller herb community, with limited effects of N enrichment or the forest land-use history. Two-way interactions between treatments and incorporating intraspecific trait variation (ITV) did not yield additional inference on community height change. Contrastingly, community SLA differed when considering ITV along with species reordering, which highlights ITV's importance for understanding leaf morphology responses to nutrient enrichment in dark conditions. Contrary to our expectations, we found limited evidence of land-use legacies affecting community responses to environmental changes, perhaps because dispersal limitation was removed in the experimental design. These findings can improve predictions of community functional trait responses to global changes by acknowledging ITV, and subtle changes in light availability. Adaptive forest management to impending global change could benefit the restoration and conservation of understorey plant communities by reducing the light availability.

Introduction

Many global change drivers currently influence the organisation of plant biodiversity from the global down to the local scale (Gonzalez et al., 2016; McGill et al., 2015; Vellend et al., 2017). Plant communities are the local and dynamic assemblages of species that can respond to such changes in the environment (Cadotte et al., 2015; Götzenberger et al., 2012). Communities can respond to chronic global change drivers, such as atmospheric depositions of nitrogen (N) and climate change (Smith et al., 2009). Contrastingly, pulse disturbances from natural dynamics or the management of ecosystems additionally alters resources and conditions (Smith et al., 2009), for example in light availability (Bernhardt-Römermann et al., 2011; Fourrier, Bouchard, & Pothier, 2015; Kern, Montgomery, Reich, & Strong, 2013). Community responses to environmental change may further depend on legacies from previous changes and in particular those of past land-use (Perring et al., 2016). Disentangling the relative importance of these global change drivers is necessary to improve predictions of plant community development in a changing world (Laughlin et al., 2012; Moran et al., 2016; Verheyen et al., 2017).

Plant communities, in terms of composition and functional trait distributions, can develop progressively in response to chronic global change drivers (Smith et al., 2009). This progressive response commences with individualistic physiological responses to environmental change (Chapin & Shaver, 1985; Smith et al., 2009). Following these individualistic responses, species in communities can “reorder”, so that different species gain in dominance at the expense of other species in the community (Smith et al., 2009). Finally, species can be lost or gained from the local species pool via processes of migration (Anderson et al., 2011; Smith et al., 2009). This sequential community response can be estimated by assessing temporal change in functional traits of species, which can characterise the ecological strategies of species and individuals (Funk et al., 2017; Garnier, Navas, et al., 2016b; Violle et al., 2007). Functional traits consequently vary between species, but also within species due to local adaptation or plasticity in expression depending on the growing environment (Lajoie & Vellend, 2015, 2018; Valladares et al., 2006). This intraspecific trait variation (ITV) can have important contributions to functional trait change in plant communities (Albert et al., 2011, 2010; Bolnick et al., 2011; Siefert, 2012a), especially through nonlinear relationships or complex interactions between environmental drivers (Fajardo & Siefert, 2018; Moran et al., 2016). ITV, together with species reordering, can then jointly influence the effects of plant communities on long-term ecosystem functioning (Guittar et al., 2016; Harte et al., 2015; Suding et al., 2008).

Ecosystem responses can depend on important previous environmental changes (Ogle et al., 2015), such as persistent legacies of past land use (Bürgi et al., 2017; Perring et al., 2016). Land-use legacies are any alterations in resources and conditions of terrestrial ecosystems brought about by previous land use, and together with processes of dispersal, selection, drift and speciation can steer communities and ecosystem functions onto trajectories of change (Foster et al., 2003; Perring et al., 2016). Furthermore, land-use legacies often enforce lagging ecosystem dynamics, so that ecosystems with a slower development have more potential to be influenced by their effects (Bürgi et al., 2017). Effects of land-use legacies are consequently well-documented in temperate forests, and on community dynamics in the herbaceous understorey layer in particular (Abadie et al., 2018; Brudvig et al., 2013; Hermy & Verheyen, 2007; Newbold et al., 2015; Verheyen et al., 1999).

Forest land-use history can drive the understorey community development and trait distributions for decades to centuries through processes of dispersal limitation and competitive exclusion (Brunet, De Frenne, et al., 2012a; De Frenne, Baeten, et al., 2011; Naaf & Kolk, 2015; Naaf & Wulf, 2012). Characteristic understorey species are typically slow colonisers which constrain presence to ancient forest (Baeten, Davies, Verheyen, Van Calster, & Vellend, 2015; De Frenne, Baeten, et al., 2011). Ancient forest can be defined as continuously forested land since the earliest reliable land-use maps, not to be confused with unmanaged or pristine “old growth forest” or “primary forest” (see McMullin & Wiersma, 2019). Slow colonisers have resource conservative traits, with a low leaf area to leaf mass ratio (specific leaf area, SLA) and a low height (Blondeel, Remy, et al., 2019; Verheyen, Honnay, et al., 2003). Reproduction by seed is often less important to these species, as they may favour clonal reproduction (Klimešová et al., 2016; Verheyen, Honnay, et al., 2003), which inhibits colonisation. These species can perform photosynthesis in dense forests, either as spring-flowering geophytes (Mabry et al., 2008; Rothstein, 2000; Tessier & Raynal, 2003) or as shade tolerant plants that persist in dark conditions (Valladares & Niinemets, 2008). Contrastingly, fast colonising species may be more abundant on afforested old fields or “post-agricultural forest” (De Frenne, Baeten, et al., 2011; Verheyen, Honnay, et al., 2003). These fast colonisers can have resource acquisitive traits with a tall stature and high SLA (Beckman et al., 2018; Díaz et al., 2016; Thomson, Moles, Auld, & Kingsford, 2011; Verheyen, Honnay, et al., 2003). Fast colonisers can benefit from a persistent legacy of large soil phosphorus stocks (P) as a result of prior fertilisation (De Schrijver, Vesterdal, et al., 2012), which can vary due to intensity of previous agricultural practices (Blondeel, Perring, et al., 2019; Macdonald et al., 2012). Beyond edaphic site properties, past land use can determine forest understorey development which may be further modulated onto different trajectories in response to environmental change (Perring, Bernhardt-Römermann, et al., 2018; Perring, Diekmann, et al., 2018).

Among a myriad of anthropogenic global change drivers (Fuhrer et al., 2016; Ripple et al., 2017; Steffen et al., 2015; Waters et al., 2016), three main environmental change drivers could interactively affect community development trajectories of forest understoreys in addition to the forest land-use history (Gilliam, 2007). First, intensifying forest management can increase the light availability at the forest floor by breaching canopy shade (Valladares et al., 2016). Plant community theories predict that increasing light availability can modulate the herb layer development trajectory in sites where N and P are less limiting to plant growth (Grime, 2001; Jabot & Pottier, 2012; Tilman, 1988), which has been observed in temperate forest understories (Baeten, Vanhellemont, De Frenne, De Schrijver, et al., 2010; Hedwall & Brunet, 2016). Second, this increase in light availability can accelerate effects of climate warming so that herbaceous communities come to resemble those of warmth-adapted communities (Bjorkman et al., 2018; De Frenne et al., 2015; Helsen et al., 2017; Henn et al., 2018; Hoeppner & Dukes, 2012). The increasing light availability may also alter soil microbial communities and enhance their activity which can release nutrients for plants (Ma et al., 2018; Ni et al., 2018). Third, N enrichment from atmospheric deposition (Bobbink et al., 2010) can enhance plant growth (Fraterrigo et al., 2009; Hejcman et al., 2012; Siefert & Ritchie, 2016), and can change community composition by increasing the abundance of nitrophilic species when light availability is high (Dirnböck et al., 2017, 2014; Gilliam et al., 2016; C. A. Walter et al., 2017).

Here we assess trajectories of forest understorey communities in response to treatments of enhanced light availability, warming and N enrichment. We performed these treatments in a full-factorial experiment on artificially assembled plant communities comprising species with varying ecological strategies, on ancient and post-agricultural forest soils. This setup allows us to assess effects of agricultural legacies on communities unaffected by dispersal limitation (see also Barker et al., 2019). We hypothesise:

1. **Community response driven by species.** Species can reorder in response to environmental treatments, leading up to changes in community composition and potentially species richness declines (given that increases are not possible in the experimental design). We expect contrasting development trajectories of communities due to species-specific responses to the forest land-use history and treatments of light addition, warming and N addition.
2. **Community response in functional traits.** Species reordering can change functional traits averaged across the community. In addition, intraspecific trait variation (ITV) can co- determine functional trait distributions in communities. We expect that communities change towards higher dominance of taller plants with higher SLA where environmental limitation is alleviated due to multiple environmental treatments. Concretely, we examine evidence for two-way interactions between light addition, N enrichment, warming and an agricultural land-use history on change in community-mean plant height and SLA.

Materials and Methods

We performed a full-factorial experiment with 384 experimental units (mesocosms) to disentangle effects of forest land-use history, enhanced light availability, nitrogen addition and warming on plant communities assembled from fifteen common European herbaceous species. The experiment started in April 2016 and data collection for this analysis ended in August 2018. We measured plant height values from 3445 individual plants and characterised SLA using 1125 leaf samples at the halfway point in the experiment (the growing season of 2017). We calculated two community weighted mean trait metrics on these values; one metric incorporates intraspecific trait variation (ITV) and one uses a single average trait value per species.

Experimental methods

This mesocosm experiment used soil from eight environmentally contrasting regions in temperate Europe to increase the generality of the results (Blondeel, Perring, et al., 2019; Verheyen et al., 2017). We collected soil (0.1 m^3 in each forest) from eight temperate European regions across gradients of inherent soil fertility, regional phosphorus balance and nitrogen deposition (further details in Blondeel et al. 2019 and Chapter 2). Within each region, we searched for three adjacent pairs of ancient forest (in existence before 1850) and post-agricultural forest (established around 1950) with similar canopy composition, leading to 48 forest patches included in the study (Blondeel, Perring, et al., 2019).

The agricultural legacies in the post-agricultural forest soils varied along gradients of inherent soil characteristics, regional P balance and N deposition (Blondeel, Perring, et al., 2019). Overall, the soils from post-agricultural forest had 0.4 units higher pH (+0.41 ± 0.16 standard error (S.E.)) than in ancient forest (mean ancient pH = 4.82 ± 0.33 S.E.). Soil C:N was lower (-1.49 ± 0.24 S.E.) in post agricultural forest than in ancient forest (mean ancient C:N = 13.9 ± 0.94 S.E.). Finally, Soil P concentrations were elevetad in post agricultural forest for total P (+118 mg kg^{-1} ± 60 S.E.) and Olsen P (+13 mg kg^{-1} ± 5.5 S.E.) compared to ancient forest (mean ancient total P = 347 mg kg^{-1} ± 64.5 S.E.; mean ancient Olsen P = 15.3 mg kg^{-1} ± 4.5 S.E.). The collected soils were classified in three groups using cluster analysis according to inherent soil fertility (texture and calcareous properties). These soil groups were either i) "Eutrophic" which are rich in clay and carbonates with high pH (6.6-7.1), ii) "Oligotrophic" which are high in sand and low in pH (4 – 5) or iii) "Mesotrophic" with intermediate fertility, siltier textures and intermediate pH (5-5.6) (see Blondeel et al., 2019). These soil groups are relative terms to categorise inherent soil fertility within our samples, and should not be used to compare outside of our population (further details in Blondeel et al. 2019 and Chapter 2). We use the region as a random effect term and the categorical soil type variable as a covariate when analysing data using linear mixed-effects models (see section “Data analysis”).

These soils were used in a large mesocosm experiment, performed in a fenced area of the Aelmoeseneie forest (Gontrode, Belgium) since April 2016 (further details in Chapter 3). The mesocosms consist of five species with differing colonisation capacities and forest habitat use. We compiled a pool of fifteen species commonly found in temperate European forests (Chapter 3) and divided this species pool according to three emergent groups that are linked with a species’ colonisation capacity (De Frenne, Baeten, et al., 2011; Verheyen, Honnay, et al., 2003) and whether a species can be considered as a forest specialist. The species in this pool can be characterised by the colonisation capacity index (CCI), where more negative values indicate a higher capacity to successfully colonise into post-agricultural forests (Verheyen, Honnay, et al., 2003). The continuous CCI scale exists from -100 to +100, where -100 means that the species only occurred in post-agricultural forest, while +100 means that the species was only found in ancient forest. At the time of planting, each mesocosm consisted of two poor colonisers and forest specialists, two intermediate colonisers of post-agricultural forest and not strictly forest specialists, and one fast-colonising nitrophilic species (see Chapter 3 for details on community assembly). These combinations of five species originated from twelve fixed species combinations that were randomly assembled (Chapter 3). These twelve species combinations were repeated four times over the 48 soil samples in each two level factorial combination of light x warming x nitrogen addition (48 soil samples x 8 factorial combinations = 384 mesocosms). In total, we planted 7680 (384 mesocosms x 20) individuals of fifteen species (512 individuals per species).

We have applied three two-level environmental treatments in a full-factorial design since April 2016 (Chapter 3). Ongoing treatments consist of (i) nitrogen enrichment; (ii) experimental warming and (iii) enhanced light availability. The first treatment is N enrichment with an additional 50 kg N ha^{-1} yr^{-1} eq., further referred to as treatment “N”. We applied the N treatment as four seasonal pulses per year, each time adding 0.25 L of a 2.01 g L-1 solution of NH_4NO_3 to each mesocosm and rinsing the leaves with 0.25 L of demineralised water. The control mesocosms received 0.5 L of demineralised water during each of the seasonal additions. The second treatment consisted of experimental warming (referred to as treatment “T”) using 75 cm-wide open top chambers (De Frenne et al., 2010). This treatment significantly raised the air temperate between 1°C and 2° C in spring 2017 and 2018, but with insignificant temperature effects when the forest canopy closed towards the end of May (Chapter 3). The third treatment, light addition (referred to as “L”) adds around 25 µmol $m^{-2}s^{-1}$ PAR to the ambient light conditions (below 10 µmol $m^{-2}s^{-1}$ under fully closed canopy) by use of two 18 W fluorescent tubes suspended 75 cm above ground level of each plot (Chapter 3). This increment in light availability can be associated to a small forest gap, and could increase carbon assimilation rates in typical forest understorey plants by three times (Rothstein & Zak, 2001). The lights were programmed to follow the natural photoperiod throughout the year and did not significantly affect air temperatures (De Frenne et al., 2015).

Summarising the design of the 384 mesocosms, we have eight regional origins of soil (classified in three groups according to inherent soil fertility), with three ancient and three post-agricultural forest soils (LU) in each region (48 soil samples), using two-level factorial treatments of N enrichment (N), warming (T) and light (L) addition (8 combinations).

Plant trait and community measurements

We measured plant height and SLA in the second growing season of the experiment, from March 2017 to September 2017, at the time of each species' estimated biomass peak (Chapter 3, Table 3.1, see Chapter 4 for methods). These measured trait values can thus denote the fitness of each species in the different treatment combinations (Garnier, Navas, et al., 2016b), 16 in total for T,L,N,LU combinations. We consequently have multiple trait measurements within each species in the different treatment combinations, but not at a different time point, so that ontogeny, growth or changing trait values within individuals are not taken into account. We chose not to measure reproductive traits (i.e. seed mass) as proposed in the common leaf-height-seed ecological strategy scheme (Laughlin et al., 2010; Westoby, 1998). Several herbaceous species in our pool favour clonal reproduction, so that seeding is often limited in these species (Klimešová et al., 2016). Sexual reproductive traits within and between species can also have ambiguous responses to multiple environmental drivers (Garnier, Navas, et al., 2016b). Furthermore, these reproductive traits can be intensive to correctly measure and quantify given our large numbers of test plants (Pérez-Harguindeguy et al., 2013).

We were able to measure plant height on the 3445 (45%) survivors of the 7680 initially planted individuals (see Chapter 3 and 4). Vegetative plant height (unit: m) was measured as the shortest distance between ground level and the upper boundary of main photosynthetic tissues. This means that we solely measured foliage height, not inflorescence or seeding height (Pérez-Harguindeguy et al., 2013).

We measured average specific leaf area (SLA) as the one-sided area of multiple leaves from a single species in each mesocosm, divided by the oven-dry mass and expressed in mm^2 mg^{-1} (Pérez-Harguindeguy et al., 2013). We collected maximally eight leaves of each species per mesocosm and no more than two per individual to avoid excessive damage to any individual plant. We collected fully expanded leaves from adult plants (which emerged in growing season of 2017), but not including a petiole where present. We avoided leaves with pathogen or herbivore damage. Because SLA is strongly affected by light intensity, we only sampled outer leaves (Pérez-Harguindeguy et al., 2013). The leaves were collected in flasks with a few drops of deionized water under high CO_2 conditions (breathing in flask before sealing) to maintain turgor and prevent wilting. Prior to measuring leaf area, we gently patted dry each leaf. The projected area of the leaves was measured with the *Easy leaf area free* mobile application (Easlon & Bloom, 2014) within two hours of picking the leaves, after taking a picture of the leaves with a mobile phone (13 MP camera). This open-source software package can estimate green leaf area in a RGB photograph by counting the total amount of green pixels and converting this into an area measurement by counting red pixels from a 4 cm^2 calibration square provided in the image. After the area measurement (mm^2), each leaf sample was dried in an oven at 65°C for 48 hours to ensure constant mass upon weighing (in mg).

In order to calculate an abundance weighted mean trait value for the community, we estimated species cover in all mesocosms at seven instances between May 2016 and August 2018. In 2016, we visually estimated the percentage cover of each species in each mesocosm in the field during the second week of May (11th May, 1 month since start of experiment), in the first week of July (6th July, after 3 months) and in the second week of September (12th September, after 5 months). To spread this labour intensive effort more efficiently over the year, we subsequently modified our method. With this modification, we first measured the total vegetation cover since 2017 using digital images. We took digital RGB photographs of the mesocosms perpendicular to the ground surface at two time points in 2017, during the first week of May (4th May, after 13 months) and the second week of August (11th August, after 16 months). We repeated this process in 2018, during first week of May (7th May, after 25 months) and the second week of August (10th August, after 28 months). These images were analysed using the *Canopy Area* tool that measures green pixels of vegetation and recalculates this into a cover percentage (Easlon & Bloom, 2014). From these images, we visually estimated each species share of the total vegetation cover.

Data analysis

All data analysis was performed in R (R Core Team, 2019). We used the package *nlme* (Pinheiro et al., 2018) to calculate linear mixed effects models. We therefore applied a consistent random effects structure including separate random effects of region (8 levels), species combination (12 levels), the group of four mesocosms (“Plot”, 96 levels) and soil type (3 levels). The focal predictor variables for community change (species reordering and/or functional signature) are warming (T), enhanced light availability (L) and N enrichment (N) with the potential for two-way interaction with the forest land-use history (LU).

In the first hypothesis, we test whether warming (T), enhanced light availability (L), N addition (N) and the forest land-use history (LU) altered community trajectories in terms of species loss and species reordering as main effects. We first tested whether species richness changed from May 2016 until August 2018 at the seven recorded time points, depending on two-way interactions of year (Year) with T, L, N, LU and of season (spring vs summer) in two-way interaction with T,L,N,LU. We therefore applied a consistent random effects structure including separate random effects of region (8 levels), species combination (12 levels) and the group of four mesocosms i.e. “plot” (96 levels). We tested these predictors in one generalised linear mixed effects model (Poisson error distribution) with our invariable set of random effect terms using the *glmmPQL* function from the *MASS* package (Venables & Ripley, 2002), with a t-test provided by the summary output (estimates and standard errors, Table A6.1.1).

To test the species reordering over time in response to the four treatments, we performed a principal response curves (PRC) analysis (Van den Brink & Ter Braak, 1999) on the seven recorded time points between May 2016 and August 2018. We used the *prc* function in the vegan package (Oksanen et al., 2019) to perform these analyses. The principal response curve method is based on redundancy analysis (RDA) and is commonly used to assess changes in biological communities to stressors, from an eco-toxicological perspective (van den Brink, den Besten, bij de Vaate, & ter Braak, 2009). The The PRC method allows estimating which species gain in abundance at the expense of others in response to the treatment and with “time” as an explicit factor (Van den Brink & Ter Braak, 1999). The graphical output shows abundance changes over time in the control treatments transformed to a flat zero reference line (x-axis) while abundance change in the treatment is allowed to deviate from this reference. The accompanying species scores (right hand y-axis) can be multiplied with the canonical coefficients of the partial RDA (left hand y-axis), which yields the proportional increase or decrease of a species in the treatment compared to the control (*100 for a percentage change). Species with large negative scores increase with the treatment, while species with positive scores decrease in abundance relative to the control. Species scores within 0.5 distance of zero indicate little abundance change in response to the treatment. The significance ($p < 0.05$) of the PRC is tested by performing a Monte Carlo simulation (10 000 permutations) of the time series, using ANOVA (F-test) which evaluates the first axis of the partial RDA.

In the second hypothesis, we test whether cover-weighed community weighted means (CWM) of plant height and specific leaf area (SLA) change over time in response to two-way interactions of T, L, N and LU. We selected the difference between the situation in May 2016 (first spring recording) and May 2018 (last spring recording). Mid spring (end of April/early May) is the time of year with maximal community biomass with all species present, and has traditionally been the time for performing forest understorey vegetation surveys (Hermy et al., 1999; G. F. Peterken, 1974). We calculated two CWM measures from these cover abundances: one that ignored within-species variation in trait values (a “single species- single trait value” approach, CWM_{single}), and a CWM where we account for different trait values within species due to the array of growing environments in the experimental setup (CWM_{ITV}). CWM_{single} simply uses the average measured trait value of a species across all mesocosms to calculate a CWM (see Table A4.1.1 in Chapter 4), and can only change when species reorder in abundance. We weighted these species trait values by the species covers in a given mesocosm at a given time, divided by the sum of those species covers. CWM_{ITV} applies the same abundance measure, but instead uses one of 16 average trait values for each species depending on the factorial combination of the two-level T, L, N and LU treatments (16 combinations, see Table A4.1.1 in Chapter 4). CWM_{ITV} can consequently differ due to species reordering and ITV together. To infer the amount of variation that species reordering explains in community trait responses, we calculated an R^2 value between CWM_{single} and CWM_{ITV} as the squared value of the Pearson’s correlation coefficient between these two variables. The higher the R^2 value, the more that species reordering explains the community response. We calculated this R^2 value across years, and also for Year 1 and Year 3 seperately to check wether the contribution of ITV (via abundance changes) alters over time.

We then calculated community change over time as log response ratios, i.e. the natural logarithm of the CWM in May 2018 over the CWM in May 2016, for both CWM_{single} and CWM_{ITV}. We analysed these as a function of two-way interactions between warming (T), light availability (L) and N enrichment (N) and the forest land-use history (LU, ancient forest as intercept). We performed backwards variable selection using the *drop1* function (R Core Team, 2019), where in each step the interaction with the highest p-value on a Chi-squared test is dropped from the model. This initial model structure includes all main effects (T, L, N, LU), all two-way interactions (T:L, N:LU, L:LU, T:N, T:L, L:N) , a fixed-factor covariate Soil type (3 levels), and the random effect terms (Region (8), species combination (12), plot (96)). The final model, after stepwise deletion of insignificant interactions, includes all main effects and any interaction term with associated p values smaller than 0.05. We evaluated model residuals against normality with a qq-plot of the models, which all had acceptable agreement between observed and theoretical quantiles within two standardized quantiles around zero. We also checked the model plots of residuals vs fitted values to check for inconsistencies given the factors included in the model, which we did not observe. We checked for independence of residuals on treatment levels, and found no further anomalies.

Results

Looking at compositional change (hypothesis 1), we found that species richness in the mesocosms decreased as expected over the course of three years with on average one lost species per year (Figure 6.1A, Table A6.1.1). We planted the experiment at March 2016 with five species, but the mean species richness in mean May 2016 was already four species (4.1, 95% CI [3.8; 4.4]). Within-season variability occurred due to the presence of vernal species, with summer surveys having one less species on average than the spring surveys in the first year (3.0, 95% CI [2.7; 3.4]), a trend that persists in Year 2 and 3 as well (Figure 6.1A). Estimates of mean species richness and the time variables did not significantly differ on the 95% confidence interval depending on warming (T), light addition (L), N addition or the land-use history (LU) of the soil (Figure 6.1B).

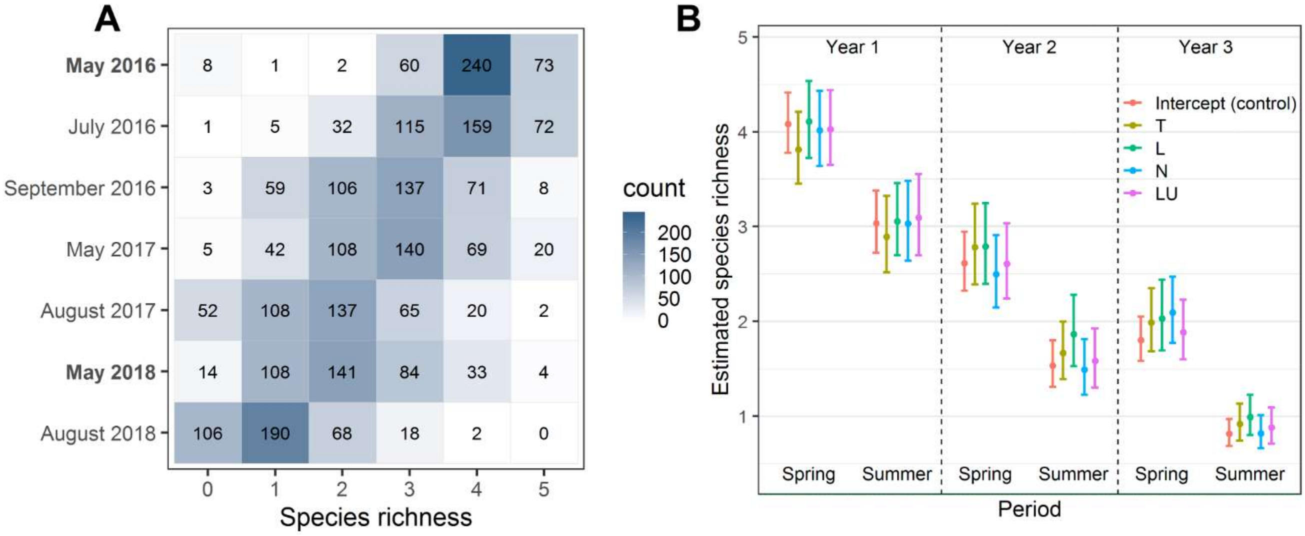

Figure 6.1. Species richness declined across all treatments, with one less species on average per year and one fewer species in summer months. Frequencies of mesocosms (A, inside the tiles) show declining species richness across all treatments over the course of three growing seasons. Summer (July – September) had one less species than spring (May) surveys, due to the presence of spring ephemerals. There are no significant differences in estimates of mean richness between main effects of treatments (B), while the temporal variation in species richness is clearly significant.

We tested the reordering of species in the treatments using principal response curves (PRC). Separate PRC of light (L), warming (T), N addition (N) and the forest land-use history (LU) treatments revealed that only the light treatment had significant effects on species reordering over time (Figure 6.2A). Warming had evidence for a minor significant effect ($p = 0.06$, Figure 6.2B), while N addition ($p = 0.97$, Figure 6.2C) or the forest land-use history did not ($p = 0.23$, Figure 6.2D). Proportional abundance changes of species in the light treatment in comparison to unlit controls showed that three species increased heavily in abundance with the light treatment. These increasing species were *Carex sylvatica* (+243%), *Galium odoratum* (+120 %) and *Polygonatum multiflorum* (+72 %). In contrast, we found that *Hyacinthoides non-scripta*, (-

38%), *Ficaria verna* (-36%) and *Anemone nemorasa* (-18%) decreased in abundance when light was applied in any treatment combination.

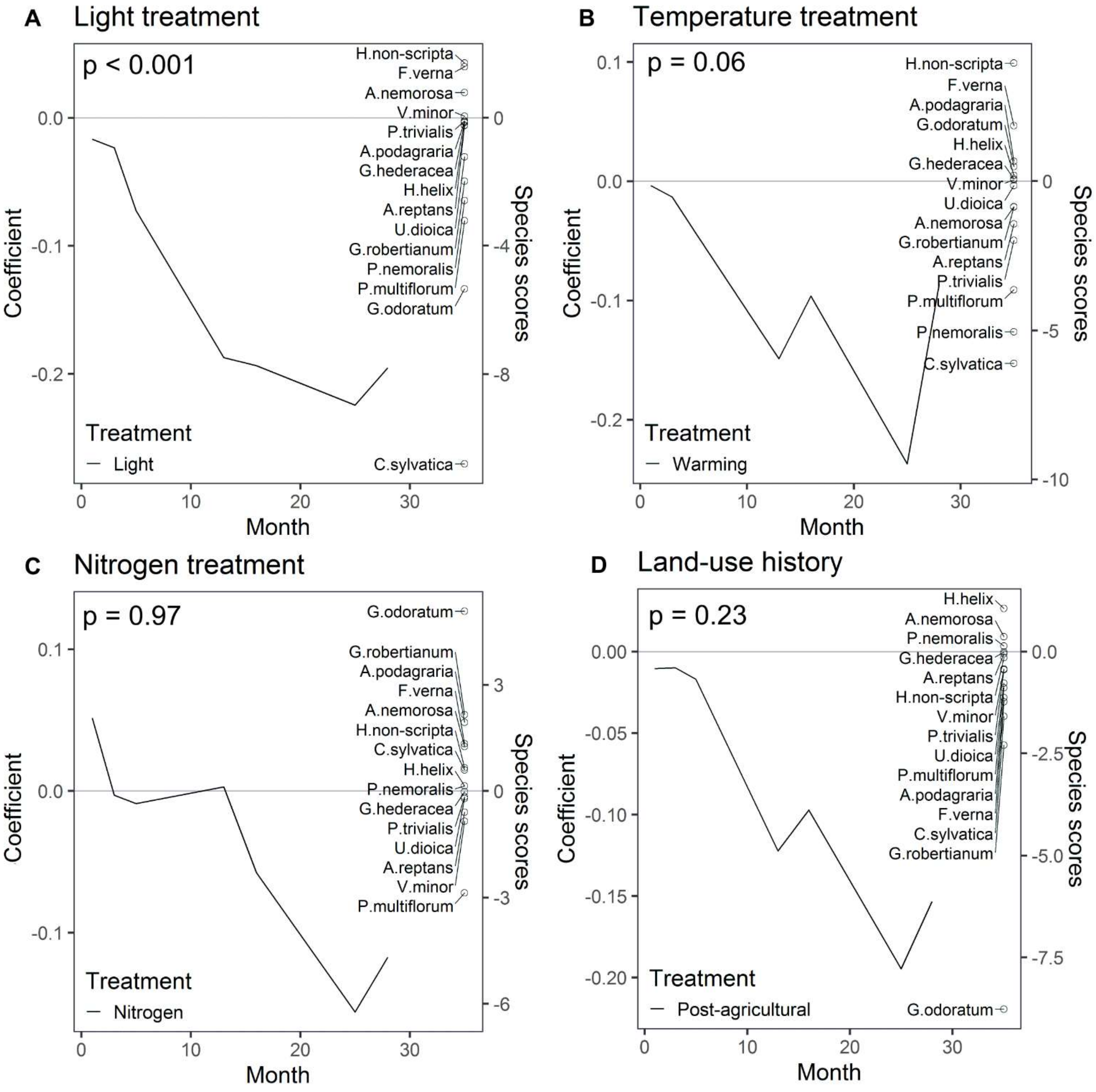

Figure 6.2. The light environment primarily determines abundance changes of species. Principal response curves of the light treatment (A), temperature treatment (B), N addition treatment (C) and the land-use history of the soil with ancient forest soil as control (D)**.** Principal response curves show abundance changes of treated vs untreated communities using partial redundancy analysis (RDA) over time. Abundance changes in the control treatments are equated to zero. Multiplying the species score (right vertical axis) by the canonical coefficient of the RDA (left vertical axis) gives the relative abundance change of a species over time. Species that increase in abundance in response to the treatment have strong negative species scores, while species that decrease in abundance within a treatment are shown in opposite directions away from zero.

Table 6.1: Proportional abundances changes (%) of each species after 25 months (May 2018) in the light treatment compared to the change in the controls in the same time period. These abundance changes are illustrations to the significant principal response curves of the light treatment found in Figure 6.2A. The proportional abundances changes are calculated as the species score multiplied by the canonical coefficient at month 25 (multiplied by 100 for percentage). Species with positive abundance change values increase with the light treatment, species with negative values increase in the unlit control conditions.

Species	Abundance change (%) due to light treatment
H.non-scripta	-38
F.verna	-36
A.nemorosa	-18
V.minor	-1
P.trivialis	2
A.podagraria	3
G.hederacea	3
H.helix	5
A.reptans	5
U.dioica	27
G.robertianum	44
P.nemoralis	58
P.multiflorum	72
G.odoratum	120
C.sylvatica	243

To test the second hypothesis, we applied a functional perspective to community change and tested log response ratios of CWM plant height and SLA in function of two-way interactions between treatments.

The plant height estimate of log RR for light is positive in the two CWM measures (Figure 6.3, Table A6.1.2) but slightly larger in CWM_{single} (+0.19± 0.04 S.E., $p < 0.001$) than in CWM_{ITV} (+0.15± 0.05 S.E $p = 0.002$). The estimate of plant height change to warming measured by CWM_{single} (+0.16± 0.04 se, $p < 0.001$) is also larger than CWM_{ITV} (+0.10± 0.05 se, $p = 0.04$). These positive plant height changes due to light and warming are also evident when looking at the CWM trait values directly rather than equating the change with the log RR (Figure 6.4A and Figure 6.4B). Overall, the shared variance between CWM_{single} (change driven by species reordering) and CWM_{ITV} (change driven by species reordering and ITV) of plant height was high (76%-) across years but increased over time ($R^2_{overall}$ 0.76; $R^2_{Year1} = 0.37$; $R^2_{Year1} = 0.84$).

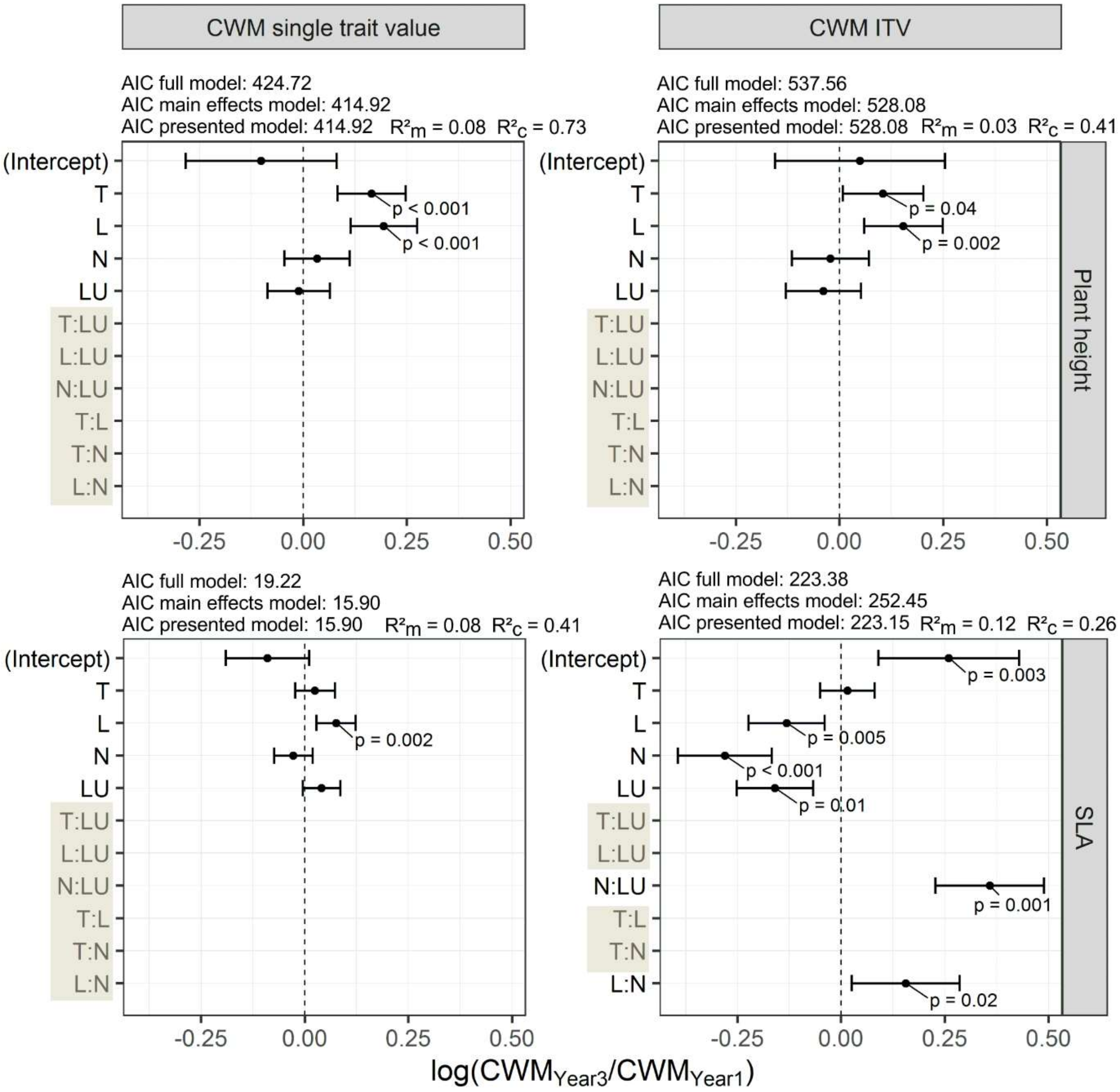

Figure 6.3. Light and warming are drivers for community plant height change over time due to species reordering, while SLA varies due to nutrient enrichment and light availability when considering ITV. Log response ratio of the community weighted mean (CWM) trait value in Year 3 over Year 1. The left panel for each trait has species reordering as the only source for CWM trait change ("single species- single trait approach"), while the right panels show the CWM value that incorporates intraspecific trait variation (ITV). The presented model was selected after backwards variable selection of the two-way interaction model of the temperature treatment (T), Light addition (L), N addition (N) and forest land-use history (LU, post-agricultural as effect relative to ancient). This presented model after variable selection includes all main effects and can include any significant interaction, which only occurred for CWM_{ITV} SLA. The comparison in AIC between the full interaction model, main effects only model and presented model (solution from selection procedure) is shown to support the model selection process. The model fit is given for the presented model, and includes fixed effects only ("marginal" R^2_m) or with random effects included ("conditional" R^2_c). P-values for factors are shown for significant effects only, see supplementary table S1.6 for detailed model summary output (including the Soil type covariate). The large difference in R^2_m and R^2_c is driven by the random effect term Community (12 levels, one for each species combination, see Table A6.1.3). This difference in R^2 shows the importance of the initial species combination for determining a CWM of a trait value.

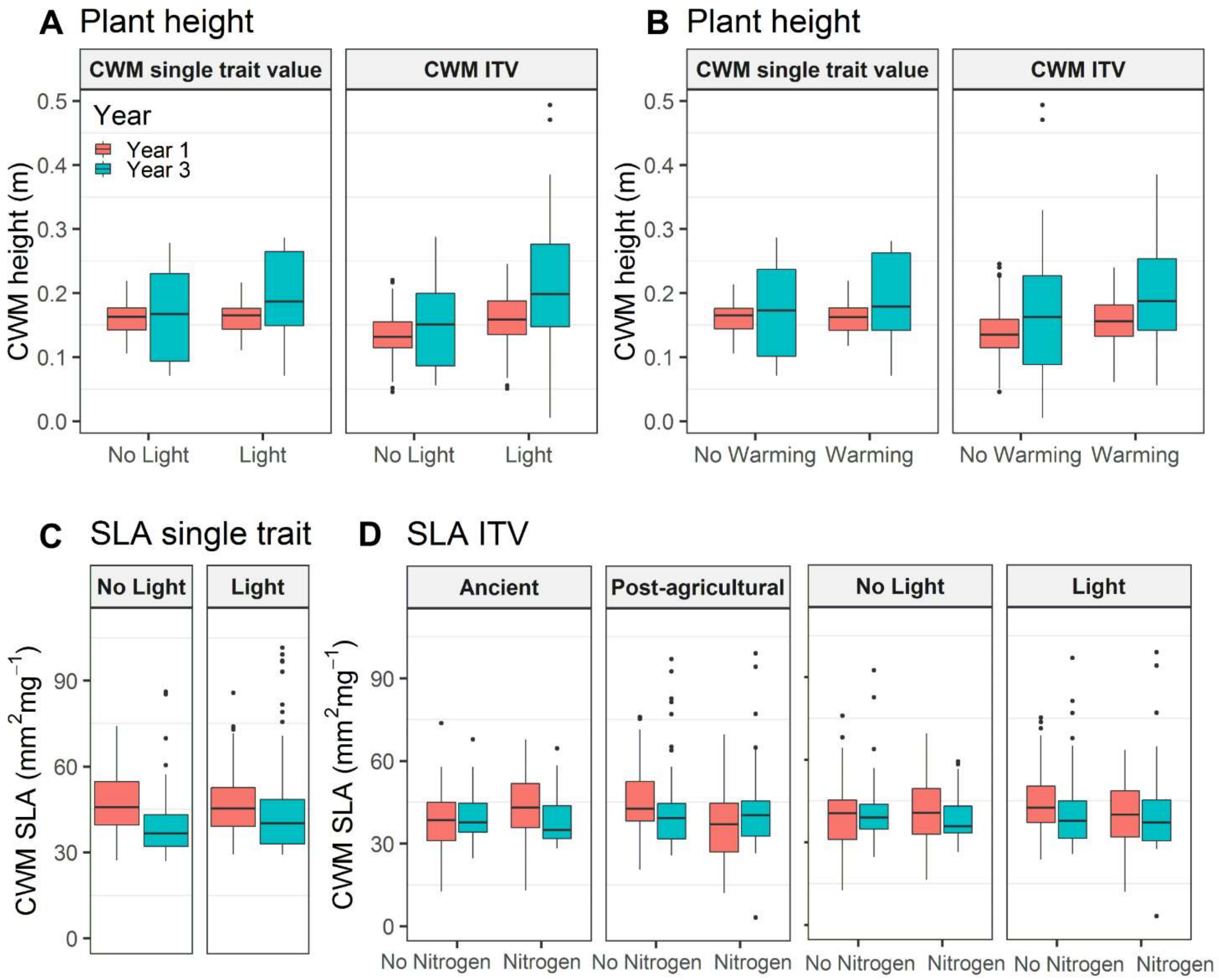

Figure 6.4. Community plant height consistently responds to light addition and warming due to species reordering, while SLA response changes with intraspecific trait variation (ITV). The boxplots on the interquartile range of CWM trait values are illustrations to the significant terms ($p<0.05$) from Figure 6.3. We found the same significant effects of light (A), and warming (B) on plant height regardless of whether ITV was taken into account. CWM SLA responded to light in a single-species single-trait approach, but driven by some outliers (C). CWM SLA response becomes more variable when ITV is considered (D) as the effect of N addition depends on the land-use history and on light availability.

$\text{CWM}_{\text{single}}$ and CWM_{ITV} of SLA yielded different significant responses to the environmental treatments (Figure 6.3, Table A6.1.2). Light had a significant positive effect on log RR of $\text{CWM}_{\text{single}}$ (+0.08 ± 0.02 S.E., $p = 0.002$), driven by some outlier points (Figure 6.4C). Contrastingly, log RR of CWM_{ITV} SLA showed a significant interaction between N addition and the forest land-use history (Figure 6.3) and another interaction between N addition and the light treatment (Figure 6.3). These interactions result from variation in CWM_{ITV} SLA at the initial survey, which converged towards the mean SLA after three years (Figure 6.4D). The variability that ITV causes in community SLA is also evident from the lower shared variance, in comparison with plant height, between $\text{CWM}_{\text{single}}$ (only driven by species reordering) and CWM_{ITV} (driven by species reordering and ITV). The shared variance between $\text{CWM}_{\text{single}}$ and CWM_{ITV} is 42 % across years ($R^2_{\text{overall}} = 0.42$; $R^2_{\text{Year1}} = 0.37$; $R^2_{\text{Year3}} = 0.68$). Consequently, 58 % of the variation in CWM_{ITV} is caused by using different trait expressions of species depending on the treatment combination. However, this importance diminishes over time, likely because abundance changes of few dominant species drive the community trait response in later years, leading to the converging pattern of CWM_{ITV} SLA in Year 3 (Figure 6.4D).

Discussion

We found that light and warming were prominent drivers for diverging plant community trajectories in our mesocosm experiment, more than N enrichment and the forest land-use history. Interactions between treatments were rarely important for explaining variation in community trait change. The light treatment modulated abundance changes in the herbaceous communities over time so that species reordered, with spring geophytes as winners in unlit conditions. Species in communities reordered towards taller plants when illumination and warming were applied. Increasing plant community height was three quarters driven by species reordering, with leftover variation accounted for by intraspecific trait variation (ITV). In contrast, responses of community SLA differed when considering ITV in comparison to the effects of species reordering only. These findings show the importance of ITV for leaf morphology responses to nutrient enrichment in dark conditions. Incorporating ITV in community SLA is thus necessary to make accurate predictions in future global change scenarios, but less important for plant height. An important and unexpected result of our experiment was the limited role of interactions between global change treatments and agricultural legacy in community development, given the expectation of evidence for its importance in observational studies (Hermy & Verheyen, 2007; Newbold et al., 2015; Perring et al., 2016).

Light availability drives development trajectories of understorey communities

Multiple studies have shown that light availability is an important trigger for community responses to nutrient enrichment and global change (Hautier et al., 2009). This viewpoint stems from classical theoretical ecology, where competition for light is predicted when N and P are less limiting, which can lead to species loss (Chapin & Shaver, 1985; Freckleton & Watkinson, 2001; Grime, 2001; Lonsdale & Watkinson, 1982; Tilman, 1990). We did not find species loss due to treatments in our experiment, but rather an average loss across all treatments of two species after three growing seasons. Instead of nutrient availability, light was by far the limiting resource due to the dense tree canopy in our experimental site, which influenced abundance changes in the communities. While the PAR increments in the enhanced light availability treatments were subtle, they could be enough to already increase carbon assimilation rates in these understorey species by a third of the light saturation point (Rothstein & Zak, 2001). The importance of climate-buffering canopy shade is indeed recognised to be key in understanding understorey plant ecology in the face of global change (De Frenne et al., 2019; Landuyt et al., 2018; Valladares et al., 2016; Zellweger et al., 2019).

We found a larger abundance of taller plants in response to light availability and warming, which follows a recurring trend in plant communities across the globe (e.g. Bjorkman et al., 2018). Short-term experimental data show that enhanced light availability in temperate forests can accelerate plant responses to warming by steering the community towards taller warmth-adapted species (De Frenne et al., 2015). Similar community trajectories have also been observed over the course of decades using forest vegetation resurveys (Bernhardt-Römermann et al., 2015; Perring, Bernhardt-Römermann, et al., 2018; Verheyen et al., 2017). Furthermore, this trend of a taller vegetation with warming was not restricted to forest areas, but has also been observed in the faster warming tundra biome (Bjorkman et al., 2018; Myers-Smith et al., 2019). The height increase in tundra communities is attributed to species turnover rather than intraspecific trait variation (Bjorkman et al., 2018; Steinbauer et al., 2018), which matches the observations in our forest understorey experiment. Changes in other traits of tundra communities, including SLA, were reported to lag behind the predicted rates of change (Bjorkman et al., 2018; Myers-Smith et al., 2019). We too observed this unpredictability in community SLA due to a large importance of ITV, especially the first year of the experiment. Species reordering alone led to a minor positive response of community SLA to illumination (but driven by some outliers), while the community SLA that accounts for ITV responded positively to N addition on post-agricultural forest soil and with light addition. This discrepancy in community SLA response due to ITV can occur in dark temperate forest, as light and nutrient acquisition are two different aspects of resource acquisition that influence SLA within and between species (Firn et al., 2019; Smart et al., 2017).

Our results suggest that ITV is important for understanding short-term and local community responses to environmental change (Albert et al., 2011; Moran et al., 2016), but depending on the trait of interest. Functional traits that can have complex responses to resource availability should consider ITV on the community level, such as in our observed patterns of SLA. Using a single mean trait value for each species may however be used to calculate a community trait response for traits when species turnover dominates the community response, such as for plant height. This approach is popularised by virtue of large collaborative trait databases (Kattge et al., 2011; Kleyer et al., 2008; Muscarella & Uriarte, 2016; Pérez-Harguindeguy et al., 2013). In our experiment, we could characterise the driving species for the community plant height response. These were *Carex sylvatica*, *Polygonatum multiflorum* and *Galium odoratum*, which increased in abundance with light addition. These species are characterised as relatively tall species in our species pool (see Table A4.1.1 in Chapter 4), but they are not considered as fast growing resource acquisitive species but rather typical of ancient forest (Wulf, 1997). These shade-tolerant species also show higher intraspecific SLA values in low light conditions for more optimal light acquisition (Blondeel et al., conditionally accepted; Liu et al., 2016; Valladares & Niinemets, 2008). Incorporating ITV on top of species reordering can thus improve predictions of future plant communities, especially with the acknowledgment of the role of light availability in community trait responses to global change.

Interacting environmental change and land-use legacies: only time can tell

We designed this multi-factor experiment to disentangle potential interactive effects of enhanced light availability, warming, N enrichment and the forest land-use history on development of forest understorey communities. Multiple examples of such interactive effects on development of understorey communities consequently exist. Warmth-loving species can increase in abundance in bright and warm forest gap conditions, with light as the accelerating driver for this warming response (De Frenne et al., 2015). Likewise, the dominance of nutrient demanding species can increase under enrichment of N and P when light availability is high (Fraterrigo, Pearson, & Turner, 2009; Gilliam et al., 2016; Holmes & Matlack, 2017; Siefert & Ritchie, 2016; Walter et al., 2016). We thus had expectations of interactive effects between the treatments in our experiment given the previous evidence.

Against our expectations, this short-term mesocosm experiment barely revealed any interactive effects between the multiple treatments on community responses. This lack of interaction on the short-term does not imply that such interactive effects are not important to understorey community development in response to global change. It rather shows the complementary of experimental research to long-term vegetation resurveys (Perring, Bernhardt-Römermann, et al., 2018; Perring, Diekmann, et al., 2018; G. Verstraeten et al., 2013) or mechanistic modelling approaches (Dirnböck et al., 2017; Landuyt et al., 2018). Long-term experiments, vegetation resurveys and modelling are perhaps better suited to unravel such long-term interactive effects between global change drivers on understorey communities (Luo et al., 2011; Verheyen et al., 2017). Most probably, interactions between the treatments in this experiment could emerge after prolonged time when resources and conditions other than light become more limiting (Oliver & Morecroft, 2014).

Besides the short-term nature of the experiment, the unexpected lack of a land-use legacy effect on community development could be particularly driven by the factorial experimental approach and the designed community assembly. We acknowledge that a binary land-use history classification increases orthogonality but might obscure nuances in legacy effects (Bürgi et al., 2017), as the agricultural legacy could rather exist on a gradient related to the intensity of previous agricultural practices. We performed a post-hoc analysis to investigate this possibility (see Appendix 6.2 using the difference in bio-available phosphorus (Olsen P) between pairs of post-agricultural and ancient soil origins as a proxy for the intensity of prior fertilisation practices (Blondeel, Perring, et al., 2019). This additional analysis confirmed a general lack of a land-use legacy effect linked to past fertilisation on community trajectories. For now, our full-factorial experiment with introduced species may not be able to reveal such long-term effects on community development. This experimental design does however allow the interpretation of land-use legacy effects without dispersal limitation as a driver for community assembly (see also Barker et al., 2019), as we bypassed dispersal by planting communities in selective combinations. The disentangled view on community development points towards an important role of light availability, followed by warming, in short-term community response to global change.

Reducing light availability is key to restore forest understoreys under global change

Similar experimental results have shown that the establishment of introduced woodland flora is not reduced in post-agricultural forest on the short-term (Barker et al., 2019) nor on longer terms (Baeten & Verheyen, 2017), when forest canopies are relatively undisturbed. Our findings thus support the notion that adaptive management to global change can restore small statured spring-flowering geophytes with high conservation priority in post-agricultural forest, but only when light is a limiting resource to enable coexistence with other species (Vandekerkhove, Verheyen, & De Keersmaeker, 2011; Verheyen, Honnay, et al., 2003). A structurally rich forest with a dense shrub layer can easily develop on former agricultural land over the course of a few decades to provide such a suitable forest microclimate (De Schrijver, Vesterdal, et al., 2012; Fridley & Wright, 2018; Sercu et al., 2017). The only remaining condition for settlement of slow colonising spring-flowering geophytes then to alleviate dispersal limitation (Baeten et al., 2015; Barker et al., 2019). This can be achieved by increasing habitat connectivity between source populations (Brunet & von Oheimb, 1998; Naaf & Kolk, 2015) or by reintroductions from the local species pool (Bucharova, 2017; Drayton & Primack, 2012; Godefroid et al., 2011; Seddon, 2010). The latter action resembles the community assembly in our experiment, with mixtures of slow colonising spring-flowering geophytes and fast colonisers on both ancient and post-agricultural forest soils. Transplanting or sowing spring-flowering geophytes is, however, currently a management strategy that has rarely been put into practice beyond experimental forest ecology (e.g. Baeten & Verheyen, 2017; De Frenne, Brunet, et al., 2011; Lajoie & Vellend, 2018; Van Der Veken, Rogister, Verheyen, Hermy, & Nathan, 2007; Yang et al., 2018).

Conclusions

We found that light availability and warming reordered species towards a taller herbaceous community, with limited effects of N enrichment and the forest land-use history. Interactions between treatments and ITV were unimportant for the plant height response. Contrastingly, community SLA differed when considering ITV along with species reordering, which highlights ITV's importance for understanding responses of leaf morphology to nutrient enrichment in dark conditions. Incorporating ITV on top of species reordering can thus improve predictions of future plant communities. Furthermore, subtle differences in light availability can yield large community trait responses to global change. This insight provides perspectives for adaptive management to global change, and demonstrates that reducing light availability is key for understorey restoration and conservation in a changing world.

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Appendix 6.1. Supporting figures and tables

Table A6.1.1. Model predictions for species richness in function of time effects (Year and season) and treatment (T, L N and LU). The Season has two levels (spring and summer) with “Spring” (April – May- June) the intercept value and “Summer” (July – August – September) the effect. Year is a categorical variable with three levels (2016 – 2018). We used a generalised linear mixed effects model and Poisson error distribution, with richness ~ T*season + L*season + N*season + LU*season + T*Year + L*Year + N*Year + LU*Year + season*Year + (1|Region) + (1|Com) + (1|Plot). The estimates in this table are from the back-transformed exponentiated Poisson regression coefficient $e^{(intercept+e \qquad)}$. The lower and upper 95% confidence intervals around the estimate of the mean species richness, and were calculated as $e^{\left(incpt+est+\sqrt{\left(1.96*SE_{incpt}\right)^2+(1.96*SE_{est})^2}\right)}$.

Year	Season	Treatment	Mean richness	Lower CI	Upper CI
1	spring	Control	4.1	3.8	4.4
1	summer	Control	3.0	2.7	3.4
2	spring	Control	2.6	2.3	2.9
2	summer	Control	1.5	1.3	1.8
3	spring	Control	1.8	1.6	2.1
3	summer	Control	0.8	0.7	1.0
1	spring	T	3.8	3.5	4.2
1	summer	T	2.9	2.5	3.3
2	spring	T	2.8	2.4	3.2
2	summer	T	1.7	1.4	2.0
3	spring	T	2.0	1.7	2.3
3	summer	T	0.9	0.7	1.1
1	spring	L	4.1	3.7	4.5
1	summer	L	3.1	2.7	3.5
2	spring	L	2.8	2.4	3.2
2	summer	L	1.9	1.5	2.3
3	spring	L	2.0	1.7	2.4
3	summer	L	1.0	0.8	1.2
1	spring	N	4.0	3.6	4.4
1	summer	N	3.0	2.6	3.5
2	spring	N	2.5	2.1	2.9
2	summer	N	1.5	1.2	1.8
3	spring	N	2.1	1.8	2.5
3	summer	N	0.8	0.7	1.0
1	spring	LU	4.0	3.7	4.4
1	summer	LU	3.1	2.7	3.6
2	spring	LU	2.6	2.2	3.0
2	summer	LU	1.6	1.3	1.9
3	spring	LU	1.9	1.6	2.2
3	summer	LU	0.9	0.7	1.1

Table A6.1.2. Estimates from the final model that was retained from the backwards selected variable selection. The initial model was for each trait and metric: CWM ~ T + L + N + LU + Soil type + T:L +T:N +L:N + L:LU+ L:N + T:LU + (1|Region) + (1|Com) + (1|Plot). The intercept here is the condition with eutrophic soil type, control treatment for T, L, N and on an ancient forest soil. LU therefore gives the estimate for a post-agricultural forest soil.

Trait	Metric	Term	Estimate	S.E.	T - statistic	P -value
Plant height	**CWM single trait value**	(Intercept)	-0.10	0.09	-1.10	0.274
		T	0.16	0.04	3.92	**<0.001**
		L	0.19	0.04	4.72	**<0.001**
		N	0.03	0.04	0.82	0.411
		LU	-0.01	0.04	-0.29	0.782
		Soiltype Meso	-0.07	0.08	-0.81	0.418
		Soiltype Oligo	-0.12	0.09	-1.30	0.194
	CWM ITV	(Intercept)	0.05	0.10	0.47	0.639
		T	0.10	0.05	2.11	**0.036**
		L	0.15	0.05	3.17	**0.002**
		N	-0.02	0.05	-0.47	0.639
		LU	-0.04	0.05	-0.85	0.419
		Soiltype Meso	-0.04	0.10	-0.40	0.690
		Soiltype Oligo	-0.06	0.10	-0.59	0.555
SLA	**CWM single trait value**	(Intercept)	-0.09	0.05	-1.75	0.081
		T	0.02	0.02	1.00	0.320
		L	0.08	0.02	3.14	**0.002**
		N	-0.03	0.02	-1.18	0.241
		LU	0.04	0.02	1.74	0.082
		Soiltype Meso	-0.13	0.05	-2.70	**0.007**
		Soiltype Oligo	-0.15	0.05	-3.11	**0.002**
	CWM ITV	(Intercept)	0.26	0.09	3.00	**0.003**
		T	0.01	0.03	0.44	0.658
		L	-0.13	0.05	-2.83	**0.005**
		N	-0.28	0.06	-4.85	**<0.001**
		LU	-0.16	0.05	-3.41	**0.011**
		Soiltype Meso	-0.13	0.08	-1.73	0.085
		Soiltype Oligo	-0.17	0.08	-2.09	**0.038**
		L:N	0.16	0.07	2.34	**0.020**
		N:LU	0.36	0.07	5.36	**0.001**

Table A6.1.3. Random effect terms of the log response ratio models (see Table A6.1.2). The random effect terms were evaluated with ANOVA on F-test, for Region (8 levels), Community (12 levels) and Plot (96 levels).

	Log RR CWM height						**Log RR CWM SLA**					
	Single trait			**ITV**			**Single trait**			**ITV**		
term	**Df**	**f**	**p**	**Df**	**f**	**p**	**Df**	**f.value**	**p**	**Df**	**f**	**p**
Region	7	0.386	0.911	7	1.074	0.377	7	1.566	0.141	7	0.831	0.561
COM	11	11.782	**<0.001**	11	15.823	**<0.001**	11	5.872	**<0.001**	11	0.089	1.000
Plot	95	0.273	1.000	95	0.187	1.000	95	0.051	1.000	95	0.004	1.000

Appendix 6.2. Intensity of past land use as driver for change

Methods

In an additional post-hoc analysis, we set out to check whether the intensity of the past agricultural land-use history would determine the magnitude of community trajectory change under the different treatments. We used the bio-available P fraction, measured as Olsen P, as a proxy for the intensity of prior agricultural land-use. We selected Olsen P for this purpose, as it can remain elevated due to past fertilisation for multiple decades after agricultural abandonment. Furthermore, we found that Olsen P in our forest soils depended on the agricultural intensity at the regional level (see Blondeel et al 2019, Chapter 3).

We calculated the difference in Olsen P of a given post-agricultural soil origin with the Olsen P of its paired ancient forest soil origin (see Blondeel et al. 2019 and Chapter 3 for soil origins and paired approach). We had eight regions included in the study, with three pairs within each region, so that we have 24 of these Olsen P differences (further referred to as Δ P). This Δ P value is thus an indicator for the intensity of prior agriculture, with large Δ P reflective of a larger fertilisation legacy in post-agricultural forest soil.

We calculated the community change over time using the previously calculated log response ratio of CWM trait values (log RR) between May 2016 and May 2018 (see “Data analysis” in this Chapter). For each combination of warming (T), light addition (L) and N addition (N), we subtracted the log RR of the post-agricultural soil origin with the log RR of its paired ancient origin (Δ log RR). Our expectation was that this Δ log RR as function of Δ P could depend on another treatment (i.e. with different slopes). This interactive effect would thus show that the magnitude of a response to a treatment depends on a gradient in land-use legacy, as opposed to evaluating its interaction on the categorical land-use history factor (LU, ancient vs post-agricultural) with ANOVA.

Results

We did not find any dependence of plant height Δ log RR on the Δ P gradient, with no differences in slopes depending on light addition (Figure A6.2.1), N addition (Figure 6.2.2) or warming (Figure A6.2.3). For SLA, we found that N addition had a positive effect on of Δ log RR when trait variation is taken into account (CWM_{ITV}). This N treatment effect on the change difference in post-agricultural and ancient forest soil did not depend on the Δ P gradient, so that there is no difference in slope (Table A6.2.1, Figure A6.2.2). The pattern in this N treatment effect on CWM_{ITV} of SLA thus aligns with our result of an N x LU interaction in the main analysis (Figure

6.3). This was the only land-use history dependent effect that we found in the main analysis, so that our analysis here using a land-use history gradient does not deliver any additional evidence.

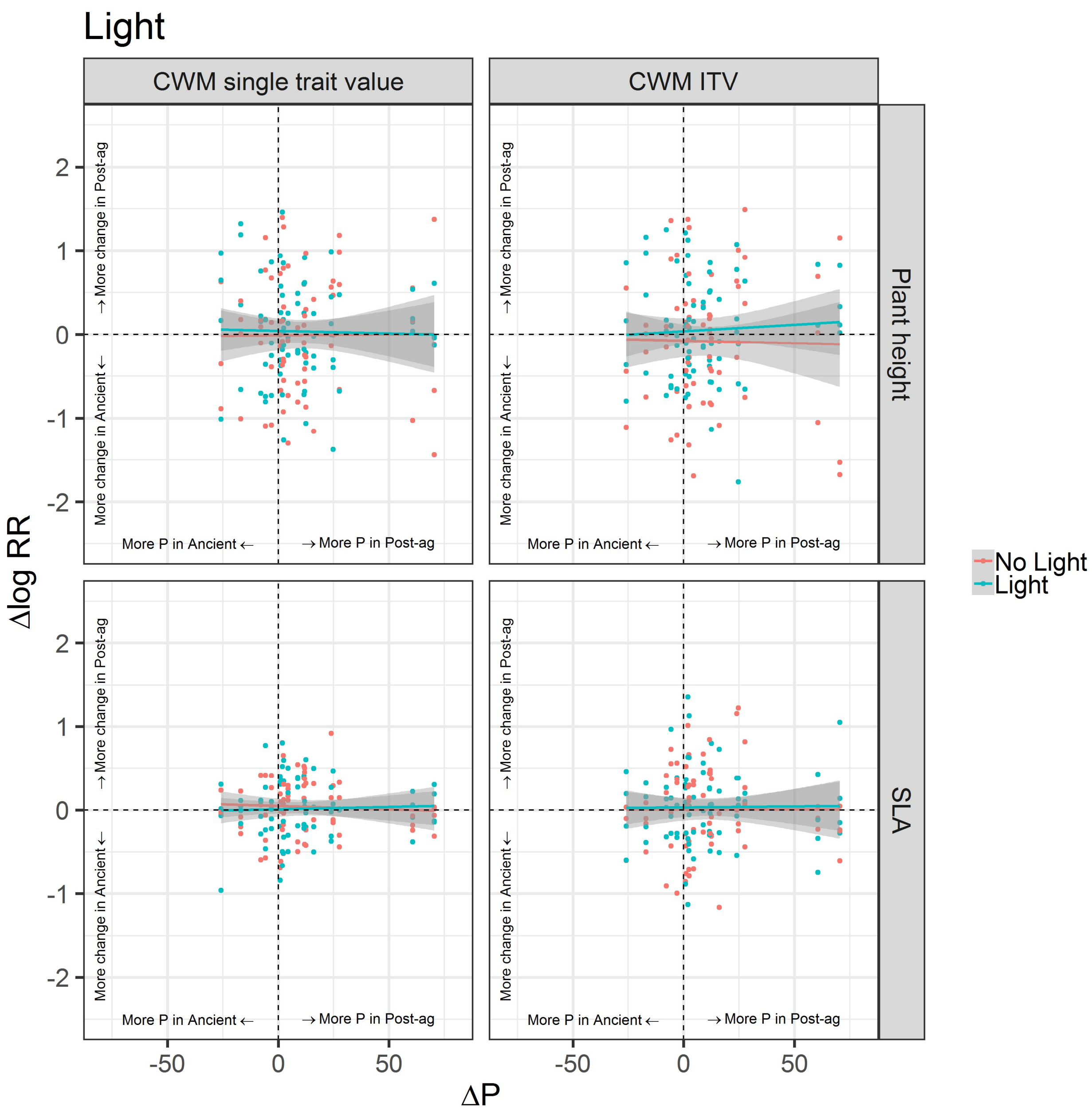

Figure A6.2.1.The difference in log RR between post-agricultural and ancient forest soil (Δ log RR) did not depend on the land-use history gradient (Δ P) within the light treatment.

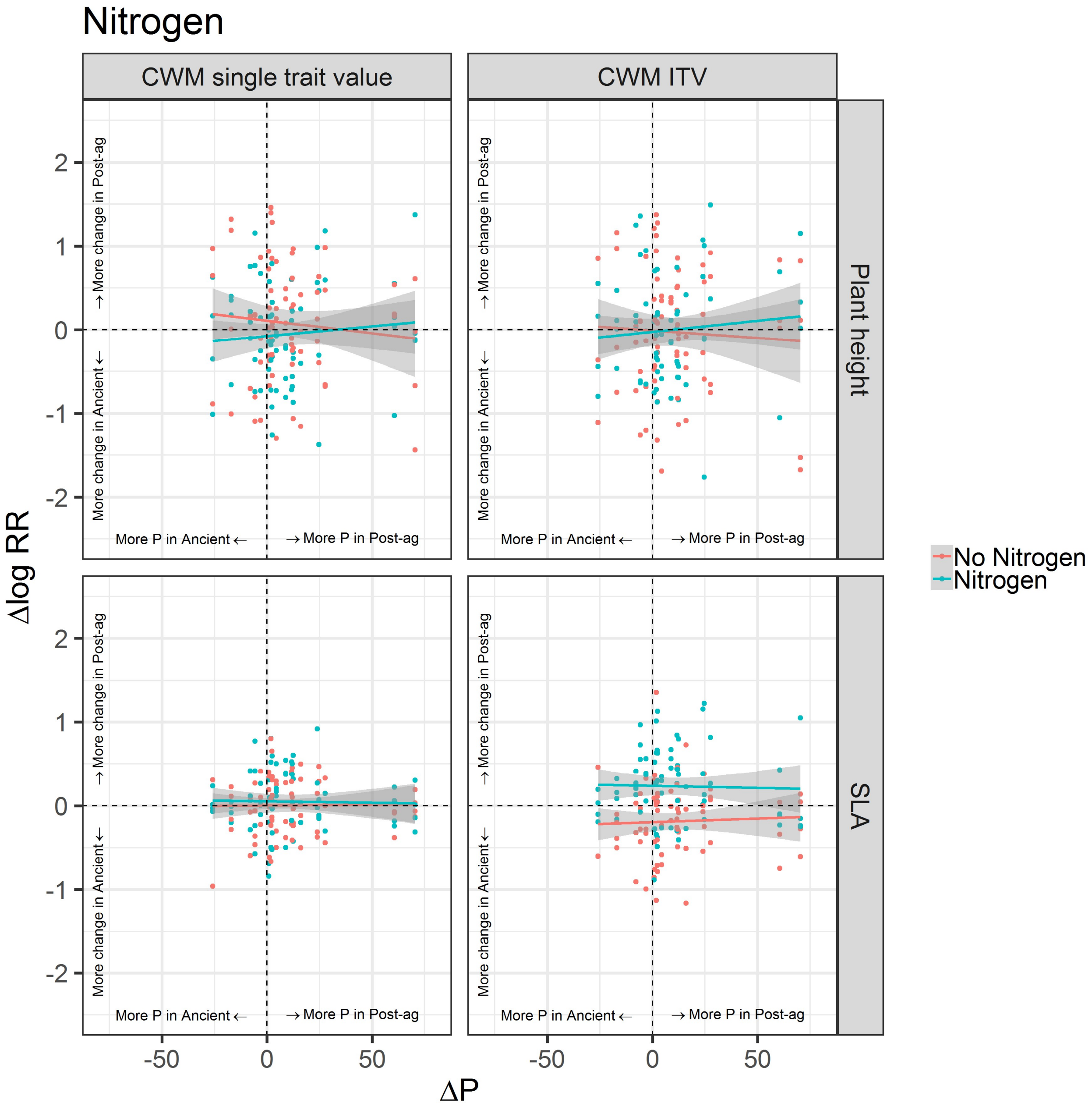

Figure A6.2.2. The difference in log RR between post-agricultural and ancient forest soil (Δ log RR) did not depend on the land-use history gradient (Δ P). N addition did however increase Δ log RR, but with a negligible slope of the effect depending on the Δ log P gradient. This effect translates into the significant interaction that we found between N addition and the categorical land-use history variable in the main analysis (see Table 6.2 and Figure 6.3)

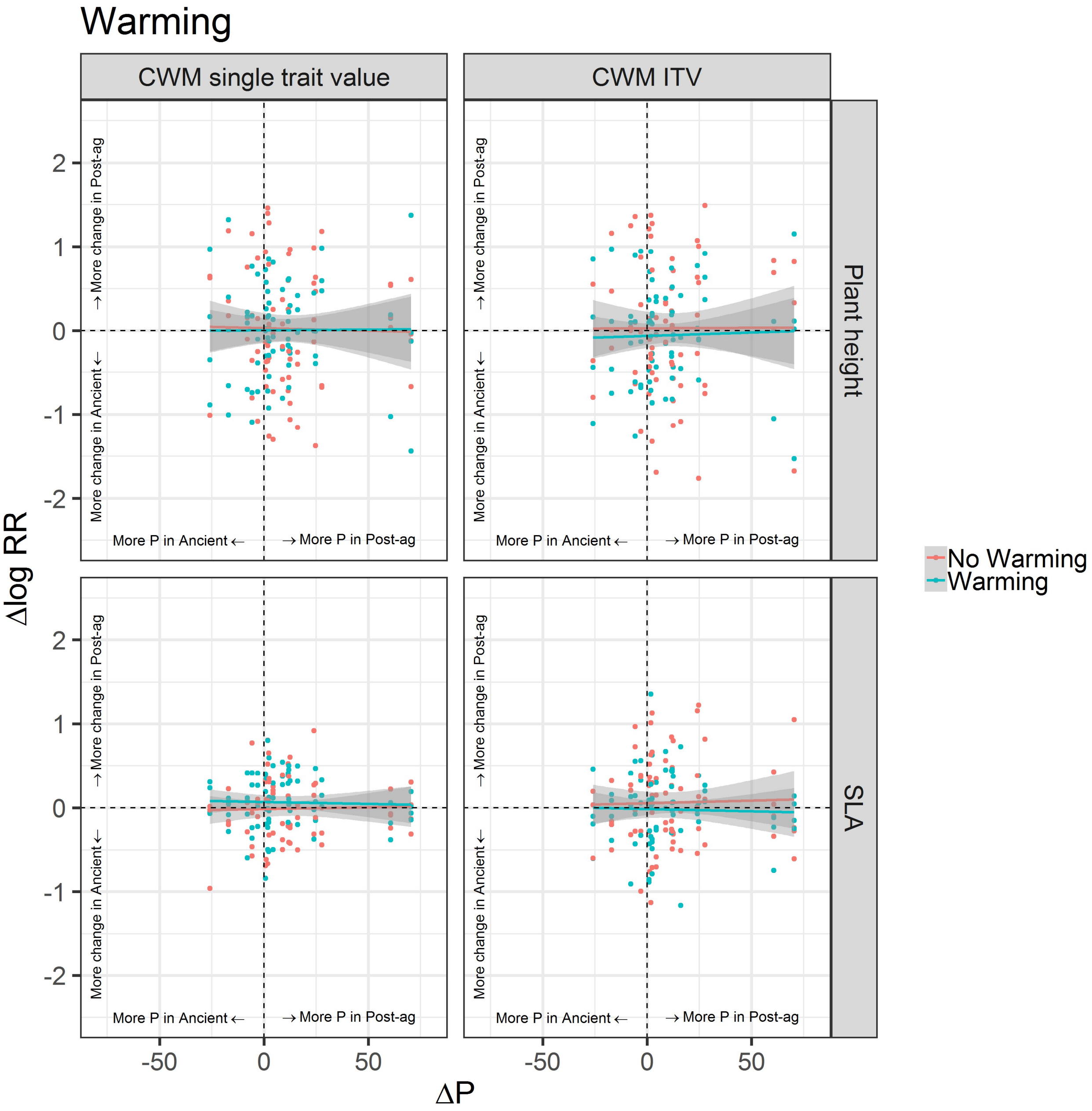

Figure A6.2.3.The difference in log RR between post-agricultural and ancient forest soil (Δ log RR) did not depend on the land-use history gradient (Δ P) within the warming treatment.

Table A6.2.1. Estimates of Δ log RR for plant height and SLA, on a single species single trait approach and with incorporating ITV. The interaction of Δ P with Nitrogen addition, Light addition and Warming were taken into account. Δ P is a slope value for the control (situation of no warming, no light addition and no N addition). The interaction is hence a new slope value (relative to the intercept) within each treatment.

Trait	**Metric**	**Term**	**estimate**	**Standard error**	**t statistic**	**p value**
Plant height (m)	CWM single trait value	(Intercept)	0.10	0.12	0.87	0.39
		Δ P	0.00	0.00	-0.67	0.51
		Nitrogen	-0.19	0.12	-1.60	0.11
		Light	0.05	0.12	0.39	0.70
		Warming	-0.03	0.12	-0.30	0.77
		Δ P:Nitrogen	0.01	0.00	1.14	0.25
		Δ P:Light	0.00	0.00	-0.11	0.91
		Δ P:Warming	0.00	0.00	0.19	0.85
	CWM ITV	(Intercept)	-0.02	0.13	-0.14	0.89
		Δ P	0.00	0.01	-0.69	0.49
		Nitrogen	-0.01	0.12	-0.12	0.91
		Light	0.12	0.12	0.93	0.35
		Warming	-0.10	0.12	-0.80	0.42
		Δ P:Nitrogen	0.00	0.01	0.95	0.34
		Δ P:Light	0.00	0.01	0.53	0.60
		Δ P:Warming	0.00	0.01	0.05	0.96
SLA ($mm^2\ mg^{-1}$)	CWM single trait value	(Intercept)	-0.02	0.07	-0.35	0.72
		Δ P	0.00	0.00	0.04	0.97
		Nitrogen	0.05	0.06	0.81	0.42
		Light	-0.04	0.06	-0.62	0.54
		Warming	0.09	0.06	1.35	0.18
		Δ P:Nitrogen	0.00	0.00	-0.22	0.82
		Δ P:Light	0.00	0.00	0.47	0.64
		Δ P:Warming	0.00	0.00	-0.36	0.72
	CWM ITV	(Intercept)	-0.19	0.08	-2.24	0.03
		Δ P	0.00	0.00	0.31	0.76
		Nitrogen	0.43	0.08	5.50	< 0.001
		Light	0.05	0.08	0.60	0.55
		Warming	-0.06	0.08	-0.72	0.47
		Δ P:Nitrogen	0.00	0.00	-0.35	0.73
		Δ P:Light	0.00	0.00	0.16	0.87
		Δ P:Warming	0.00	0.00	-0.62	0.54

Image: Sofie Verstraelen

Chapter 7

General Discussion

We performed a large mesocosm experiment (384 experimental units) to disentangle effects of light availability, nitrogen (N) enrichment, experimental warming and agricultural land-use history on the development of forest understorey communities. We collected soil from 24 pairs of ancient (in existence before 1850) and post-agricultural forests (established on arable land since 1950) in temperate Europe (Chapter 2). These 48 soils provided the source material to plant mesocosms. These mesocosms consisted of communities of herbaceous species with varying colonisation capacities, planted on the soils with a distinct land-use history. This planting scheme bypasses dispersal limitation in species, as we planted slow and fast colonisers on soils with both an ancient and post-agricultural land-use history. This scheme allows focusing on the biogeochemical aspect of agricultural land-use legacy effects on plant community development. We applied two-level experimental treatments of warming, N addition and enhanced light availability to these mesocosms in a full-factorial approach (Chapter 3). We used this set-up to investigate how the altered resources and conditions can have combined effects on individualistic plant responses (Chapters 4 and 5) that could scale up to a community trait response (Chapter 6). We expected that individualistic plant responses to environmental treatments could depend on the land-use history, and that such combined species responses would shape the community response. Against our expectations, we did not find interactive treatment effects depending on the land-use history, neither on individualistic nor on community responses. Effects of multitple environmental treatments were simply additive, i.e. the sum of the separate treatment effects. The coming sections first cover the altered resources and conditions engendered by the treatments, before discussing their effects on the individual plant level, up to the community.

Altered resources and conditions

Agricultural land-use legacy

Combining soil data from 24 paired sites of ancient and post-agricultural forests across eight European regions successfully elucidated consistent legacies of past land use (Chapter 2). As expected, post-agricultural forest soil had an overall higher available P concentration, higher pH, and lower C:N ratio compared to ancient forest. The magnitude of these legacies varied along gradients of soil types, P nutrient management on the regional level, and N deposition. Regions with many intensive livestock farms and high N deposition, such as Flanders, had much stronger agricultural legacies than regions with less intensive agriculture (eg. Estonia, see also Powers et al., 2019). We could thus reconcile previously contrasting legacies (Compton & Boone, 2000; Macdonald et al., 2012; McLauchlan, 2006) by acknowledging the regional environmental context.

Experimental treatments of enhanced light availability, warming and N enrichment

The enhanced light availability treatment contributed to an alleviation of light limitation below the dense tree canopy. Photosynthetic active radiation (PAR) in the ambient light conditions, under a fully developed canopy, was below 10 µmol $m^{-2}s^{-1}$ (Chapter 3). To put this low value in perspective: a typical sunny day in temperate areas can yield about 1300 µmol $m^{-2}s^{-1}$ in open field conditions, but with a light saturation point for photosynthesis at 500 µmol $m^{-2}s^{-1}$ (Rothstein & Zak, 2001). The addition of 25 µmol $m^{-2}s^{-1}$ with the fluorescent tubes could disproportionately alleviate the light stress to about a third of light saturation. This disproportionality arises from the non-linear response of carbon assimilation to PAR, with the largest increment in assimilation rates before 100 µmol $m^{-2}s^{-1}$ in typical forest understorey plants (Rothstein & Zak, 2001). Artificial lighting can thus have important effects on the ecology of plants in dark conditions (Bennie, Davies, Cruse, & Gaston, 2016; De Frenne et al., 2015).

Passive warming using open top chambers proved to be most effectively in early spring, which can benefit the development of forest understoreys. We found increasing air temperatures between 1° C and 2 ° C during March and May in the consecutive growing seasons. This degree of experimental warming treatment can provide important insights for future global change, as global warming is likely to reach 1.5°C above pre industrial levels between 2030 and 2052 (IPCC, 2018). In any scenario of future global change, springtime warming has been recognised as pivotal to understorey ecology and phenology (De Frenne, Brunet, et al., 2011; Rollinson & Kaye, 2012).

Adding the equivalent of ca. 50 kg N $ha^{-1}yr^{-1}$ in NH_4NO_3 form to mesocosms yielded very limited plant responses on both the individualistic and community levels. Important to note here is that the ambient N deposition measured below the tree canopy in the Aelmoeseneie forest (15.5 kg N ha^{-1} in 2014) already falls within the critical load range (10- 20 kg N $ha^{-1}yr^{-1}$) for temperate broadleaved forests (Bobbink & Hettelingh, 2010; van Dobben et al., 2000). It could thus be that the ambient N deposition (i.e. control conditions) is too elevated for the N treatment to induce an ecological response in the treated conditions (adding eq. 50 kg N $ha^{-1}yr^{-1}$). Furthermore, it could be highly likely that the results of this experiment would strongly depend on this high N environmental context. Forest herb community responses to environmental change may depend on N deposition intensity, as a recent resurvey study across a wide N deposition gradient has shown (Perring, Diekmann, et al., 2018). Performing this experiment in a region with N deposition below the critical load range (e.g. Southen Sweden) could thus potentially yield different results regarding N addition effects.

Individualistic plant responses to multiple global changes

We measured functional traits of the species in the mesocosm, by accounting for plant height and resource acquisitive traits (specific leaf area, leaf N content). We expected that these functional traits would respond individualistically to the environmental treatments, with a potentially accelerating response (i.e. positive interaction) due to an agricultural land-use history. We found that plant height varied more in species with higher colonisation capacity (Chapter 4), along with a larger N allocation to leaves in faster colonising species (Chapter 5). Contrastingly, specific leaf area (SLA) responded to warming and illumination in spring geophytes species and shade-tolerant species respectively (Chapter 4). Individualistic species responses occurred directly in response to specific manipulations in resources and conditions that benefited a species. These individualistic responses were indirectly exacerbated by higher community productivity (Chapters 4 and 5). In the case of N allocation to leaves, these combined direct effects of environmental changes outweighed the indirect effects by three times (Chapter 5). We barely found interactive treatment effects, so that combined effects of treatments were additive and characterised by the sum of the separate treatment effects.

Legacy and environmental treatment effects on individualistic plant responses

Agricultural legacy, warming and enhanced light availability contributed to individualistic plant responses. Plants barely exhibited individualistic responses to the N enrichment treatment. The individualistic responses were analysed regardless of species abundance, and handled intraspecific trait variation (Chapter 4) and allocation of N to leaves (Chapter 5).

The additive positive effects of enhanced light availability, warming, and agricultural legacies (in that order) together nearly doubled the vegetation cover (from 40% to 75%). This large increase of community cover in turn affected individualistic plant responses, both in within-species trait variation (Chapter 4) and with N allocation to leaves (Chapter 5). Within species, we found larger plant height values with high total vegetation cover (Chapter 4), alongside a tissue dilution in foliar N (Chapter 5). Plants commonly experience a tissue dilution in nutrients when growth rates are enhanced, which has been long described in agricultural studies (Jarrell & Beverly, 1981). Structural equation models demonstrated that increased growth in individuals mostly occurred directly in response to these treatments, with high total vegetation cover contributing to an additional increase. In any case, these indirect effects of enhanced community growth were generally three times smaller than the direct effects of light and warming on trait values (Chapter 4) and tissue N dilution directly (Chapter 5).

Forest herbs along a global fast-slow growth continuum

Patterns in intraspecific trait variation (Chapter 4) and N uptake (Chapter 5) revealed that the forest herbs in our species pool were positioned along a fast-slow growth continuum of plant form and function (Beckman et al., 2018; Díaz et al., 2016; Reich, 2014; Wright et al., 2004).

The fast colonising species were tall (Chapter 4), with high SLA and large N uptake (Chapter 5), which indicates a resource acquisitive or "fast" functional signature (Reich, 2014; Díaz et al., 2016; Figure 7.1). They showed large intraspecific variation in plant height, and were taller in communities with high total cover (Chapter 4). This plasticity in stature allows these resource acquisitive species to capture more light away from direct neighbours, and so benefit from asymmetric competition for this scarce resource (DeMalach et al., 2016; Freckleton & Watkinson, 2001). Larger N allocation to leaves, alongside a taller plant height, were thus the key traits defining resource acquisition in our forest herb species pool (Beckman et al., 2018; Díaz et al., 2016; Moles et al., 2009; Reich, 2014; Thomson, Moles, Auld, Kingsford, et al., 2011; Verheyen, Honnay, et al., 2003; Westoby, 1998; Wright et al., 2004).

Figure 7.1. Tall individuals of the resource acquisitive species *Urtica dioica* in a mesocosm with light, warming, N addition and post-agricultural soil. *Urtica dioica* could double plant height in these conditions relative to controls (Chapter 4), but the species was not always so abundant as in this example (Chapter 6).

Slow colonising forest herbs were resource conservatives, as they were “slow” in allocation of tracer N to their leaves (Chapter 5). Slow colonisers also exhibited plasticity in SLA (Chapter 4), but with responses that were linked to specific life-history strategies and performing photosynthesis in dark temperate forests. We found that early flowering geophytes increased SLA with warming. This morphological response could follow from alleviating a substantial mass investment that vernal species need to take to withstand frost periods (Kreyling, Puechmaille, Malyshev, & Valladares, 2019; Milla & Reich, 2007). Contrastingly, slow colonisers that are shade-tolerant had higher SLA in low light availability. This is a common morphological response in leaves of such species in order to alleviate light stress (Liu et al., 2016; Valladares & Niinemets, 2008). The trade-offs in leaf economics (Wright et al., 2004) that these vernal species and shade-tolerant species made to capture resources seem to have lacked in physiological responses (changing leaf N), in favour of leaf morphological responses (changing SLA).

Our experimental design elucidated that forest herbs indeed follow the global fast-slow growth continuum, when dispersal limitation is overruled and land-use legacy effects are controlled for. This conclusion may be intuitive, yet difficult to underpin with empirical evidence. Investigating how growth strategies of slow colonising herb species would change in post-agricultural forest is difficult, given that dispersal limitation restricts these species to ancient forest (Brunet, De Frenne, Holmström, & Mayr, 2012b; Verheyen, Guntenspergen, Biesbrouck, & Hermy, 2003). To date, only few introduction experiments have looked at changes in plant functional traits or growth strategies in post-agricultural versus ancient forest (Baeten & Verheyen, 2017; Barker et al., 2019; De Keersmaeker et al., 2011; Thomaes, De Keersmaeker, Verschelde, Vandekerkhove, & Verheyen, 2014; Vandekerkhove et al., 2011). Previously, it could only be assumed that slow colonizers with limited dispersal abilities also show a conservative growth strategy in response to environmental change, and would suffer environmental limitation in post-agricultural forests. In our experimental deisgn, we omitted dispersal limitation and applied environmental change treatments to mixed communities of fast and slow colonising species. We found that light limitation was equally important to all species, while past land use per se had limited influence on functional trait change in species. It thus seems lilekely that slow versus fast colonising species actually also fall along the slow versus fast growth continuum in different global change contexts.

Community responses to multiple global changes

To scale the individualistic responses of plant species to the community level, we characterised abundance changes of species due to treatments over time and associated this community change to responses of functional traits on the community level. We found that species reordering primarily determined change in plant height on the community level (Chapter 6).

Change in community plant height can thus be estimated by accounting for interspecific differences of plant height in forest herb populations. However, to scale SLA from individuals to the community, individualistic responses to variation in growing environments needed to be taken into account (Chapter 4, Chapter 6). Intraspecific trait variation (ITV) is consequently key to understand SLA change in communities over time, which is potentially true for all leaf traits that are affected by light and nutrient acquisition (see Firn et al., 2019; Smart et al., 2017).

These overarching findings imply a context-dependency in the hierarchical response framework and the associated progressive community response to environmental change (Smith et al., 2009, see Chapter 1). We found that individualistic plant responses and species reordering could interfere considerably on the short-term, largely due to the important effects that light availability had on the development of these understorey communities.

Community response to enhanced light availability, warming and N enrichment

We found that light, then warming, were the consistent drivers for diverging plant community trajectories in our mesocosm experiment. The N enrichment treatment, like the forest land-use history, had limited effects on community organisation. It might thus be safe to assert that plant growth on these soils were less limited by N and P availability, and more by light availability. This may explain why light and warming were key in affecting community development, as competition for light is predicted when N and P are less limiting (Chapin & Shaver, 1985; Freckleton & Watkinson, 2001; Grime, 2001; Lonsdale & Watkinson, 1982; Tilman, 1990).

Communities veered towards taller plants with illumination and warming, while spring geophytes hold steady in unlit conditions (Chapter 6). The increase in community height was for three quarters driven by species reordering with the leftover variation due to ITV. We were able to identify the driving species behind community trait change, in terms of species reordering, using a principal response curves analysis (Chapter 6). Tall but more resource acquisitive species (e.g. *Urtica dioica*, see Chapter 3) were of lesser importance to this community response due to their relatively low abundances in our experiment. *Galium odoratum, Carex sylvatica* and *Polygonatum multiflorum* were rather dominantly increasing in abundance with illumination and warming (Figure 7.2), and these are relatively tall species in our species pool (Chapter 4).

These shade-tolerant species also showed individualistic responses to light (Chapter 4). The SLA values of these species were higher in dark conditions than in the illuminated conditions (Chapter 4, and see Valladares & Niinemets, 2008; Liu et al., 2016). This plastic response of SLA in shade-tolerant species confounded our understanding of community SLA in response to global change (Chapter 6). Responses of community weighted mean (CWM) of SLA differed when considering ITV, in contrast to looking only at species reordering (Chapter 6). Community SLA driven by species reordering alone had a minor positive response to the light treatment. Contrastingly, community SLA with accounting for ITV was larger on post-agricultural soils that received additional N. This discrepancy in community SLA response due to ITV can occur in dark temperate forest, as light and nutrient acquisition are two different aspects of resource acquisition that influence SLA within and between species (Firn et al., 2019; Smart et al., 2017).

Figure 7.2. Three clear winner species, in terms of positive abundance change, emerged due to treatments of light addition and warming. These were *Galium odoratum (*left and right with white flowers), *Carex sylvatica* (graminoid in the centre back) and *Polygonatum multiflorum* (centre below), all relatively tall species in our species pool.

The larger abundance of taller plants in response to light availability and warming follows a recurring trend in plant communities across the globe. Community height increase has been observed in forest understories on the short term (De Frenne et al., 2015) and on the long term using vegetation resurveys (Bernhardt-Römermann et al., 2015; Perring, Bernhardt-Römermann, et al., 2018; Verheyen et al., 2017). Furthermore, this trend of a taller vegetation does not only occur within forests, but also in the faster warming tundra biome (Bjorkman et al., 2018; Myers-Smith et al., 2019). The height increase in tundra communities is attributed to species turnover rather than intraspecific trait variation (Bjorkman et al., 2018; Steinbauer et al., 2018), which matches the observations in our forest understorey experiment. Changes in other functional traits of tundra communities, including SLA, were also reported to lag behind the predicted rates of change (Bjorkman et al., 2018; Myers-Smith et al., 2019). Plant communities could thus become increasingly taller with future global environmental change across biomes.

Lacking agricultural legacy effect on community organisation

Against our expectations, and central to much observational research in temperate forest (Gilliam, 2014; Hermy & Verheyen, 2007), agricultural legacy had limited effects on community composition in our short-term experiment. Agricultural legacy did increase community productivity (Chapters 4 and 5), but with little discrimination between species so that this did not affect the composition of the community (Chapter 6). The lack of agricultural land-use history effects on community organisation did not stem from using a binary approach (i.e. ancient vs post-agricultural history), as assessing a gradient in land-use history intensity based on soil P concentration did not yield any new insights (Chapter 6).

We can thus infer a limited role of short-term legacy effects in our experimental communities, given the three years of monitoring in our experiment (as in much empirical work, Ives, 2018). However, due to time-lags in ecosystem response (Bürgi et al., 2017), it is highly possible that legacy effects can modulate community trajectories of change in our experiment on the long term (Ogle et al., 2015; Perring et al., 2016). This has been shown in a long-term understorey introduction experiment in Belgium (Baeten & Verheyen, 2017). Contrasting trajectories of herb layer species did not emerge in ancient or post-agricultural sites at initial surveys (Baeten et al., 2009; Verheyen & Hermy, 2004), but did after two decades with a spring ephemeral as a winner species in undisturbed conditions (Baeten & Verheyen, 2017). For now, the full-factorial design with introduced species may not be able to reveal long-term effects on community development. This experimental design does, however, allow interpreting land-use legacies without dispersal limitation as a driver for community assembly, in contrast to forest understorey development in the natural world (Baeten et al., 2015; Holmes & Matlack, 2018; Kolk et al., 2016; Naaf & Kolk, 2015).

Plant responses to multiple treatments: interactive vs additive effects

We designed this multi-factor experiment to disentangle potential interactive effects of enhanced light availability, warming, N enrichment and the forest land-use history on development of forest understorey communities. Effects of these environmental change drivers on understorey community composition and development are commonly entangled in the natural world (Perring et al., 2016). Multiple examples of such interactive effects on development of understorey communities consequently exist. Warmth-adapted species can increase in abundance in bright and warm forest gap conditions, with light as the accelerating driver for this warming response (De Frenne et al., 2015). Likewise, the dominance of nutrient demanding species can increase under enrichment of N and P when light availability is high (Fraterrigo, Pearson, & Turner, 2009; Gilliam et al., 2016; Holmes & Matlack, 2017; Siefert & Ritchie, 2016; Walter et al., 2016). We thus had expectations of interactive effects between the treatments in our experiment given the evidence from these prior observational findings.

Against our expectations, this short-term mesocosm experiment barely revealed any interactive effects between the multiple treatments, neither on individualistic responses nor on community responses. This lack of interaction on the short-term does not imply that such interactive effects are not important to understorey community development in response to global change. It rather shows the complementary of experimental research to long-term vegetation resurveys (Perring, Bernhardt-Römermann, et al., 2018; Perring, Diekmann, et al., 2018; G. Verstraeten et al., 2013) or mechanistic modelling approaches (Dirnböck et al., 2017; Landuyt et al., 2018). Long-term experiments, vegetation resurveys and modelling are perhaps better suited to unravel such long-term interactive effects between global change drivers on understorey communities (Luo et al., 2011; Verheyen et al., 2017). Most probably, interactions between the treatments in this experiment could emerge after prolonged time when resources and conditions other than light become more limiting (Oliver & Morecroft, 2014). Currently, light is the most limiting factor, followed by growing temperatures in early spring. Limiting nutrient avaibility is only of minor importance at this stage, given the limited plant responses to agricultural legacy and N enrichment in particular. The current observed effects of these treatments imply an additivity: a combination of multiple treatments could be described by the sum of their separate effects. Additive effects of multiple global changes on plant community properties are not necessarily rare, especially on relatively short terms (Zavaleta et al., 2003; Manning et al., 2006; Yue et al., 2017; but see Dieleman et al., 2012).

We found additivity in individualistic plant responses to specific environmental treatments (Chapters 4 and 5) and on change in community composition and traits (Chapter 6). This additivity between treatments also emerged on seasonal trajectories of productivity (estimated via total vegetation cover) in each of the sixteen treatment combinations (Box 7.1). However, the overlapping trajectories in the 16 treatment combinations at the spring peak in cover during the final year (2018) might indicate that more interactive treatment effects could emerge over time. The 4 year period of this PhD project thus constrained our understanding of long-term development trajectories of these communities, as with many short-term experiments (Knapp et al., 2012). Furthermore, it may be expected that higher vegetation cover would inhibit spontaneous establishment from colonising plants into the treated mesocosms. This may result in long-term competitive exclusion of potential colonising species (Thomaes et al., 2014).

Box 7.1. Trajectories of total vegetation cover

Responses of understorey cover to environmental change could be highly variable in the growing season, due to differences in life history and phenology of the species in the community. Tracking such detailed community trajectories by visual cover estimation is interesting to understand community trajectories over time, but labour intensive and potentially prone to observer errors. To increase efficiency and reproducibility, we decided to analyse photographs of the mesocosms in each consecutive month during the growing season (March – September) of 2017 and 2018 using the "Canopy area tool" (see Chapter 4 – 6). These results were satisfactory (Figure B7.1.1), but could not discriminate between species. We thus only have detailed measurements of total vegetation cover spanning this period.

Figure B7.1.1. Automated vegetation cover estimation. The vegetation cover in this image, estimated by the "Canopy area" tool, was 25.58 %. The recognised vegetation pixels are highlighted in bright green. The original image of this specific example was taken on March 03 2017. The mesocosm in question had a post-agricultural forest soil, with only illumination applied, and no warming or N addition

Total vegetation cover peaked in mid spring (Figure B7.1.2) and decreased steadily until September. Mesocosms that received light and warming together (light green – yellow shades) have the highest vegetation cover (+ 30 % cover). In comparison, land-use history is of minor importance to vegetation cover and N addition does not affect cover at all. The "stacked" appearance of the smoothed curves is a result of the additivity in treatment effects, particularly at peak spring in 2017. There is already more overlap between community trajectories at the spring peak in 2018. Interactive treatment effects on community properties during the spring peak might become apparent if this pattern persists in the future.

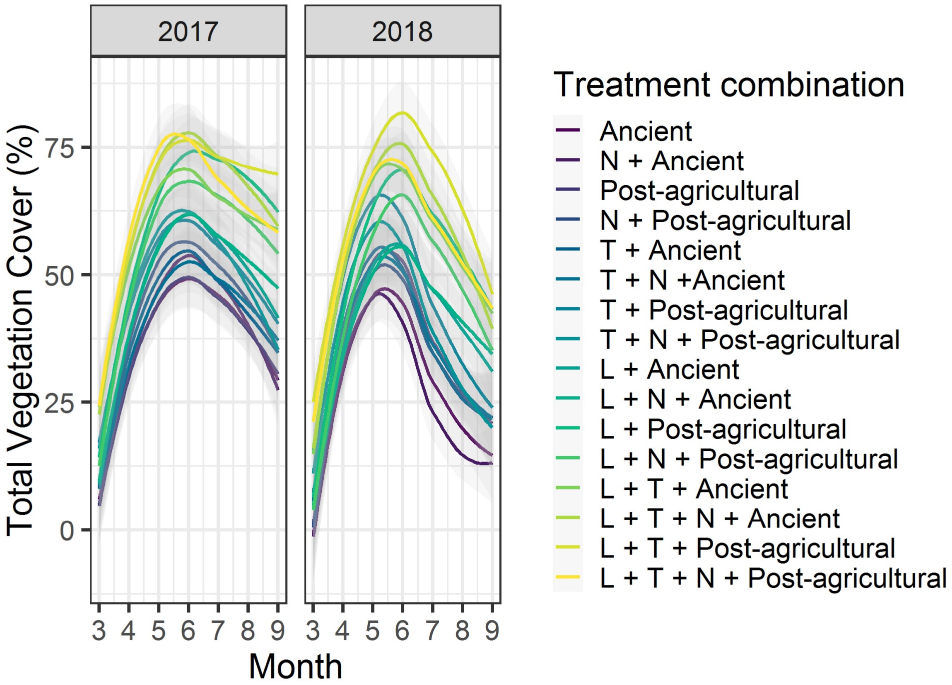

Figure B7.1.2. Seasonal trajectories of total vegetation cover (%). These curves represent the mean total vegetation cover in each of the 16 treatment combinations (Ancient or Post-agricultural, Light = L, Temperature = T, N addition = N). The cover values of all mesocosms were smoothed ("LOESS", span = 1) for presentation of the data.

Research implications for society

Conservation and restoration of the forest understorey in a changing world

To safeguard the future of forest understoreys, the adage of “protect first, restore second” holds true (Gilliam, 2018; Honnay et al., 2002; Possingham, Bode, & Klein, 2015). Forest understorey plants are of special conservation interest in temperate forests (Hermy et al., 1999), partly due to their large cultural and historical significance (Dupouey et al., 2002; McGrath et al., 2015; Szabó, 2015; Verheyen et al., 1999). This cultural heritage extends to the present, as blooming spring ephemerals are responsible for drawing attention of many recreationists in springtime (Graves, Pearson, & Turner, 2016, 2017). As rich hotspots for biodiversity and rare species, forest understoreys can feed and shelter pollinators, herbivores and detritivores (Gilliam, 2007). This functional role cements the significance of the understorey as key in nutrient and energy cycling (Gilliam, 2007; Landuyt et al., 2019; Landuyt et al., in revision.). Conserving a species rich and productive understorey is thus needed to ensure well-functioning forests of the future (Gilliam, 2016).

Forest restoration on old fields is currently oriented towards restoring tree growth, and less focused on understorey composition (Honnay et al., 2002). The understorey is then let to develop under spontaneous processes which may lag behind for centuries (Baeten et al., 2015; De Frenne, Baeten, et al., 2011; Verheyen, Guntenspergen, et al., 2003; Wulf, 1997). To increase understorey recovery rates in the future, forest managers can gain insights from experimental trait-based ecology performed in different global change contexts (Foster et al., 2016; Laughlin, 2014; Perring et al., 2015; Török & Helm, 2017).

Our experimental work supports the notion that adaptive management to global change can restore spring ephemerals in post-agricultural forest, so long as light is a limiting resource for fast growing resource acquisitive species (Baeten & Verheyen, 2017; Vandekerkhove et al., 2011). A structurally rich forest with a dense shrub layer can easily develop on former agricultural land over the course of a few decades to provide such a suitable forest microclimate (De Schrijver, Vesterdal, et al., 2012; Fridley & Wright, 2018; Sercu et al., 2017). The only remaining condition for settlement of typical woodland flora is then to alleviate dispersal limitation (Baeten et al., 2015). This can be achieved by increasing habitat connectivity between source populations (Brunet & von Oheimb, 1998; Naaf & Kolk, 2015) or by reintroductions from the local species pool (Bucharova, 2017; Drayton & Primack, 2012; Godefroid et al., 2011; Seddon, 2010). The reintroduction of understorey plant species from seed could potentially provide positive results depending on the species (Albrecht et al., 2019; Baeten, Vanhellemont, De Frenne, Hermy, et al., 2010), but is more likely to succeed if clonal material is used

(Klimešová et al., 2016). Transplanting or sowing understorey species is, however, currently a management strategy that has rarely been put to practice beyond experimental forest ecology (e.g. De Frenne, Brunet, et al., 2011; Lajoie & Vellend, 2018; Van Der Veken, Rogister, Verheyen, Hermy, & Nathan, 2007; Yang et al., 2018).

This understorey restoration strategy via dimming the light availability may, however, pose important trade-offs for other social, ecological or economical ecosystem services (Mori, Lertzman, & Gustafsson, 2017). Modern forest management actions are multifunctional, and can serve several functions other than for biodiversity protection alone. For instance, canopies are regularly opened in production forests to harvest timber and allow for tree regerneration, which can additionally increase the number of species associated with open habitats (Vangansbeke et al., 2016). With natural disturbances increasing because of climate change (insect infestations, droughts, storms, etc. see Seidl et al., 2017) , even shorter rotation times may become recommended in order to lower the number of old trees which are usually more susceptible to diseases and storms (Pennisi, 2019; Stovall, Shugart, & Yang, 2019) . Shorter rotation times may also increase the C sequestration of the forest and lock accumulated N in biomass where N deposition, is high, thereby improving its climate change mitigation role (Fowler et al., 2015; Pretzsch et al., 2014). These aspects need to be taken into account on the landscape scale, where forest stands can have a complementary management in a functionally cohesive forest habitat network (Vangansbeke et al., 2016). The recommendation of reducing light availability to support the establishment of spring-flowering geophytes may thus be mostly relevant for mesophytic deciduous forest areas with a clear protection status.

Policy to curb global change and biodiversity loss

The “Bonn Challenge” is a global initiative endorsed by the UN to restore forest on 150 million ha of degraded land across the globe by 2020, and 350 million ha by 2030 (Verdone & Seidl, 2017). This programme aims to restore forest landscapes as a whole, by increasing the numbers of trees in semi-natural and agricultural landscapes, and by securing ecological functions and benefits to human well-being (Verdone & Seidl, 2017). Among other vegetation types, the benefits of a diverse and well-developed forest understorey are currently not envisioned in this programme (Temperton et al., 2019), despite the ecological significance of the understorey to forest functioning (Gilliam, 2007).

Sadly, several authorised deforestations of ancient woodland in Belgium and Germany show that industrial benefits can still be preferred over forest conservation in the EU (Brock & Dunlap, 2018). Worse still is how the recent regime in Brazil has legalised deforestation in pristine Amazonian forest to favour agricultural expansion (Freitas et al., 2018), with little compensation whatsoever (Busch et al., 2019). Even when compensated, afforested land needs to develop for decades to centuries to reach a similar degree of ecosystem functionality as the original habitat (Griffiths, Bull, Baker, & Milner-Gulland, 2019). Decision support systems could help policy makers and land managers to identify suitable sites for adaptive forest management, conservation and restoration (Grêt-Regamey, Sirén, Brunner, & Weibel, 2017). Special attention should be given to quantify landscape connectivity and suitable abiotic conditions for the understorey (Török & Helm, 2017). The suitability of sites can be prospected and evaluated on a large scale with remote sensing techniques (Almeida et al., 2019; White, Wulder, Hermosilla, Coops, & Hobart, 2017). Policy makers thus have the tools to conserve and identify intact forests, or restore forest on degraded lands from the understorey up to the tree layer.

Beyond forest understoreys, policy action is needed to halt global species loss (Ceballos et al., 2015; IPBES, 2019; McGill et al., 2015) and avoid a collapse of ecosystems across the globe (Cardinale et al., 2012; Tilman, Isbell, & Cowles, 2014). Curbing alarming rates of global change and biodiversity loss (Ripple et al., 2017; Steffen et al., 2015) remains a top priority to secure human well-being for future generations (Díaz et al., 2018; IPBES, 2019; Millenium Ecosystem Assessment, 2005). Let us hope that the upcoming United Nations Decade of Ecosystem Restoration (2021 -2030) can stimulate the commitment of reforestation for mitigating global change during the coming years.

Further research

Current collaborations

The experimental work laid out in this thesis has already spawned additional research. We ventured to explore the importance of the forest understorey for ecosystem functioning, alongside the focus on functional trait change in understorey communities that is presented in this thesis. We therefore advertised the set-up to graduate and PhD students for performing add-on research to the ongoing work there. Two main collaborations have been established since the spring of 2017, both to explore how global change can affect the role of forest understoreys in ecosystem functioning. First, Bin Wang led a litterbag experiment to test direct and indirect effects of the understorey on decomposition of different litter types. This add-on experiment was supported by the “TeaComposition” initiative (Djukic et al., 2018), a global effort to study decomposition rates using bags of green tea (decomposes easily) and rooibos tea (recalcitrant litter). The results of this experiment are accepted for publication in Soil Biology and Biogeochemistry, and show that enhanced understorey vegetation cover can inhibit decomposition rates of labile litter in particular. The second functional role of the understorey that we explored was the recruitment of tree seedlings through the changing understorey communities. Emiel De Lombaerde planted seedlings of *Acer pseudoplatanus*, *Quercus robur*, and *Fagus sylvatica* in all mesocosms and monitored their emergence and survival for one year. These results are currently being prepared for publication.

Multiple methods can be used to analyse plant community change over time, with experimental research, long-term vegetation resurveys and mechanistic modelling being the prime approaches to infer such community responses (Luo et al., 2011; Verheyen et al., 2017). Further research efforts to integrate these approaches for assessing understorey responses to past land use and global change is underway in the PASTFORWARD project. Michael Perring is currently leading the integration of this thesis' experimental results with findings from vegetation resurveys (by Michael Perring, Sybryn Maes and Leen Depauw) and mechanistic modelling (by Dries Landuyt). In a next step, Dries Landuyt will use the collected community trait data from this thesis for calibration of mechanistic models to project forest understoreys in multiple global change scenarios. These findings will provide the basis for an innovative decision support system to benefit the public, and help forest managers predict understorey dynamics in response to management interventions in a changing world.

Future collaborations?

The experimental set-up will be preserved for the future, and will be open to use for anyone interested in understorey dynamics under different global change contexts. An important avenue to take here is to further explore the importance of the understorey to ecosystem functioning (Mori et al., 2016). Relatively straightforward plant diversity and ecosystem functioning relationships, such as for productivity, can be explored in this experimental set-up (Tilman et al., 2014). However, by introducing a fixed amount of species in the mesocosms (5), it may require more caution to understand diversity related treatment effects on belowground processes and nutrient mobilisation. A more challenging research avenue is thus to delve into the nutrient and energy cycling mediated by the understorey, to investigate interactions between communities of herbivores, pollinators and detritivores. An important aspect here is to characterise belowground nutrient and energy storage in the different herb species (Klimešová et al., 2016). It is likely that changes in community root traits might reveal potential dynamics that we have overlooked by sampling aboveground biomass (Sardans et al., 2017). We did not shed any light on belowground biotic processes, as we did not want to damage the herbaceous communities during the time that the experiment ran.

Clarifying belowground biodiversity and plant-soil feedbacks is, however, necessary to fully understand ecosystem functioning in changing ecosystems (Bardgett & Van Der Putten, 2014; Guerrero-Ramírez, Reich, Wagg, Ciobanu, & Eisenhauer, 2019). This includes the characterisation of the microbial and mycorrhizal communities that can differ due to agricultural land-use legacy (Boeraeve et al., 2018; Jo et al., 2018) and other global changes (Ma et al., 2018), and how this affects understorey composition (Veresoglou, Wulf, & Rillig, 2017). To study how plant interactions via the soil can shape the forest of the future, it can be valuable to explore allopathy in herbaceous understorey species for suppressing tree seedling growth. An example is the negative influence of foliar compounds in *Hedera helix* (one of the species in the experiment) on the recruitment of *Fagus sylvatica* (Walbott, Gallet, & Corcket, 2018). The importance of this mechanism to forest functioning might increase in the future, as *Hedera helix* is currently on the rise in forest understoreys across Europe (Perring et al. in prep.). More general, temporal dynamics in plant-soil feedbacks and their links to herbivory, life history strategy and variation in environment will be key to understand community organisation in a changing world (in 't Zandt, van den Brink, de Kroon, & Visser, 2019; Kardol, De Deyn, Laliberté, Mariotte, & Hawkes, 2013; Van der Putten et al., 2013).

A next generation of the experimental work presented in this study would benefit from studying pulse disturbances in addition to chronic disturbances (Smith et al., 2009). This research can then focus on resilience in forests, which can be defined as the recovery potential or tolerance of forests to environmental disturbances (Isbell et al., 2015; Seidl, Rammer, & Spies, 2014; Seidl et al., 2017; Smart et al., 2014). These disturbances can be either pulses, i.e. extreme events such as droughts, or chronic disturbances that alter resources and conditions in ecosystems on the long term (e.g. climate change, N deposition). Specifically, we could test whether ancient forests can tolerate heavier pulses of droughts in different global change contexts than post-agricultural forests. Resilience may be measured by the degree in which in key ecosystem functions that relate to energy and nutrient flows are affected by those pulse droughts. These ideas will be further developed in a follow-up post-doc grant proposal after this PhD study.

One of the avenues for investigating future biodiversity-ecosystem functioning relationships, be they belowground or aboveground, is to unlock the potential of big data and machine learning (Franklin, Serra-Diaz, Syphard, & Regan, 2016; Willcock et al., 2018). A venture in this PhD, in collaboration with Ruben Van De Vijver, was to apply machine-learning techniques for automated cover estimation of species in the mesocosms. This approach stumbled onto a dead end, due to complications of training the algorithm with too few "classified" images (Figure 7.3). With more time and resources, it might however be possible to characterise biodiversity-ecosystem function relationships on the community level using innovative technology. The use of unmanned aerial vehicles, multi-spectral imaging and machine learning come to mind to determine nutrient concentrations of plants and categorise the species in plant functional types (Díaz-Delgado, Ónodi, Kröel-Dulay, & Kertész, 2019; Van Cleemput, Roberts, Honnay, & Somers, 2019).

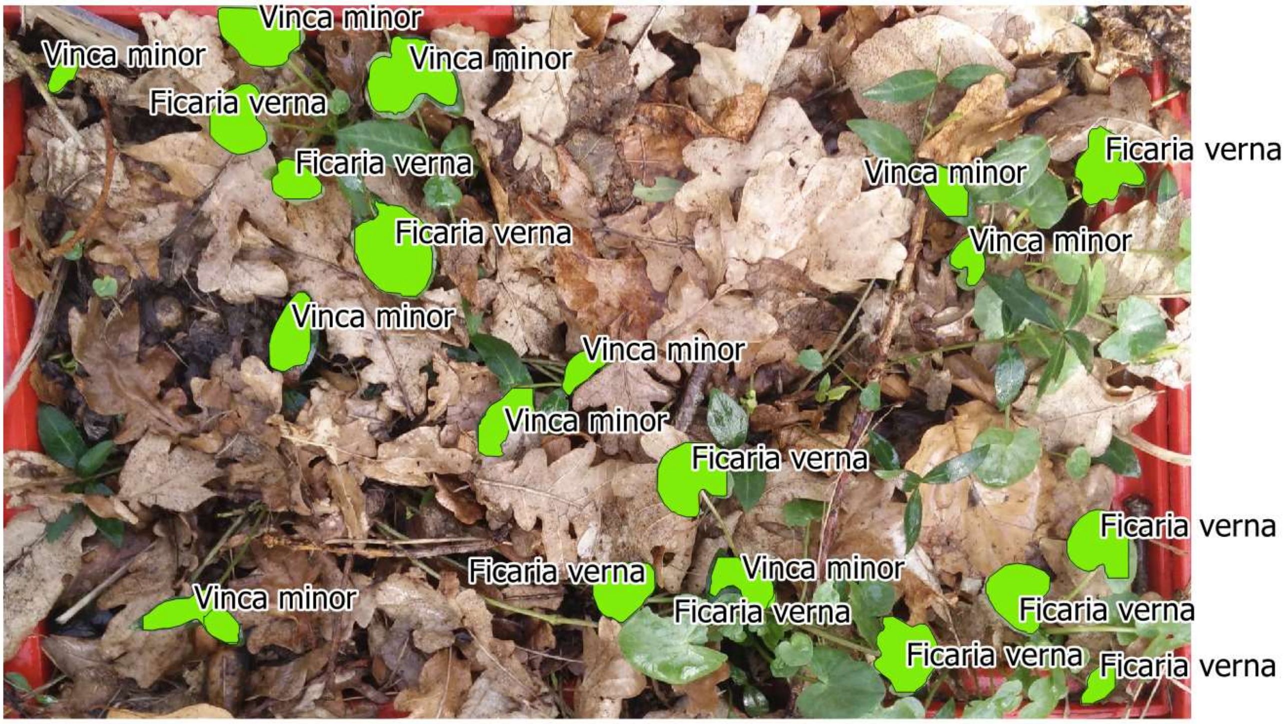

Figure 7.3. A questionable effort of the automated recognition algorithm to characterise leaves of species in the community. However, the recognised leaves in this image were nearly all from the correct species.

Long-term applied research

The research in this PhD study has built upon the work of countless vegetation ecologists. There has been a long research tradition for disentangling environmental legacies of land use from understorey colonisation via experimental empirical research (Hermy & Verheyen, 2007; Van Der Veken et al., 2007). In its simplest form, experiments have looked at biogeochemical aspects of land-use legacies (i.e. nutrient enrichment) and their effects on growth of species with different colonisation abilities in single pots (Baeten, Vanhellemont, De Frenne, De Schrijver, et al., 2010; Baeten, Vanhellemont, De Frenne, Hermy, et al., 2010; Hipps, Davies, Dodds, & Buckley, 2005). In a next step, these single-species approaches were taken to real-life forests with introduction experiments of single ancient woodland plants in post-agrcitural forests (Baeten et al., 2009; Baeten & Verheyen, 2017; Graae, Hansen, & Sunde, 2004; Verheyen & Hermy, 2004). In this thesis, we take this tradition further by introducing plant communities on soils with distict past land use, albeit in pots but in real-life forest conditions (see also recent work by Barker et al., 2019). A next logical step is to introduce whole plant communities from one forest site into the other.

Future research following this experiment can take a more applied ecological perspective for the benefit of understorey conservation and restoration in a changing world (Perring et al., 2015). A possibility is to focus on the rates of understorey recovery where light availability varies, but in real forest conditions instead of short term experiments such as performed here (Baeten, Vanhellemont, De Frenne, De Schrijver, et al., 2010; De Frenne et al., 2015; De Keersmaeker et al., 2011; Thomaes et al., 2014). Monitoring the long-term understorey recovery in combination with transplants (Baeten et al., 2009; Baeten & Verheyen, 2017; Van Der Veken et al., 2007; Verheyen & Hermy, 2004; Yang et al., 2018) can potentially unlock whether novel species interactions in the understorey occur due to effects of land-use legacies (Alexander et al., 2015).

Combining multiple site-based experiments along geographical gradients is a necessary step to scale-up the findings that we presented in this thesis (Verheyen et al., 2017, see research project FORMICA). This wide geographical and long-term approach also provides the chance to delve into social and cultural benefits of understorey recovery in multiple societal contexts. Involving social sciences into long term ecological research across multiple regions has been identified as key for solidifying the importance of ecological research in a changing world (Haase et al., 2018).

Conclusions

Multiple global environmental changes, together with legacies of previous land use, can have combined effects on development of herbaceous understorey communities. This PhD thesis aimed to unravel the relative importance of a past agricultural land use and effects of warming, enhanced light availability and N enrichment on development of herbaceous understorey communities. We performed a large field experiment to disentangle these drivers on temperate forest herb communities. We created “mesocosms” by planting mixtures of slow and fast colonising plant species on ancient and post-agricultural soils, collected across eight European regions. We can thus understand understorey development in response to a context-dependent past land use while bypassing dispersal limitation in species. This scheme allowed focusing on the biogeochemical effects that agricultural legacy had on these forest soils, which was a larger availability in phosphorus, a lower carbon to nitrogen ratio, and a higher pH.

On the short term, additive effects of light and warming were more important drivers for understorey change than effects of nitrogen enrichment or past land use. These responses persisted within species and scaled to the community level. Functional traits of species (specific leaf area (SLA), leaf N and plant height) responded to the environmental changes. Fast colonisers were tall with large N uptake, which is a functional signature that points towards resource acquisition. Contrastingly, slow colonising and typical woodland flora lacked in physiological responses (changing leaf N), in favour of leaf morphological responses (changing SLA).

On the community level, light and warming reordered species towards a taller herbaceous community but with a minor contribution of within species trait variation. However, to scale SLA from individuals to the community, variation in growing environments and associated individualistic responses needed to be taken into account. Subtle differences in light availability and air temperatures can thus yield relatively large community trait responses to global change, both within and between species. Against our expectations, these short-term experimental results showed a limited importance of N enrichment and the forest land-use history on development of herbaceous understorey communities. More time could reveal whether diverging community trajectories in response to environmental change would emerge depending on the forest land-use history. This lack of interactions on the short-term does not imply that such interactive effects are not important to understorey community development in response to global change. It rather shows the complementary of experimental research to long-term vegetation resurveys or mechanistic modelling approaches.

The findings that we have presented here can contribute to the field of global change ecology. Incorporating ITV on top of species reordering can improve predictions of future plant communities, with acknowledging that subtle differences in light availability can yield large community trait responses to global change. This insight provides perspectives for adaptive management to global change, and demonstrates that dimming light availability is key for understorey restoration in a changing world.

Image: Emiel De Lombaerde

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Curriculum vitae

Curriculum Vitae

Personal data

Name	Haben Blondeel
Address	Blekerijstraat 87 9000 Gent Belgium
Place of birth	Gent
Date of birth	Belgian
Nationality	06/01/1991
Phone	(+32) 478 23 76 42
Email	haben.blondeel@ugent.be

Education

2015 - 2019	**PhD** in Bioscience engineering forest & nature management | **Universiteit Gent**
2013 - 2015	**Master** in Bioscience engineering forest & nature management | **Universiteit Gent**
2009 - 2013	**Bachelor** in Bioscience engineering land & forest management | **Universiteit Gent**
2003 - 2009	**Secondary school** (Sciences - mathematics) | **Sint-Paulusinstituut Gent**

Scientific publications

Peer review scientific articles in A1 journals

Leen Depauw, Michael P. Perring, Dries Landuyt, Sybryn L. Maes, **Haben Blondeel**; Emiel De Lombaerde, Guntis Brumelis, Jörg Brunet, Déborah Closset-Kopp, Janusz Czerepko, Guillaume Decocq, Jan den Ouden, Radoslaw Gawrys, Werner Härdtle, Radim Hédl, Thilo Heinken, Steffi Heinrichs, Bogdan Jaroszewicz, , Martin Kopecky, Ilze Liepina, Martin Macek, Frantisek Malis, Wolfgang Schmidt, Karol Ujhazy Monika Wulf, Kris Verheyen. *Light availability and land-use history drive biodiversity and functional changes in forest herb layer communities.* Accepted at Journal of Ecology.

Sybryn L. Maes, Michael P. Perring, Leen Depauw, Markus Bernhardt-Römermann, **Haben Blondeel**; Guntis Brumelis, Jörg Brunet, Guillaume Decocq, Jan den Ouden, Sanne Govaert, Werner Härdtle, Radim Hédl, Thilo Heinken, Steffi Heinrichs, Lionel Hertzog, Bogdan Jaroszewicz, Keith Kirby, Martin Kopecky, Dries Landuyt, Frantisek Malis, Thomas vanneste, Monika Wulf, Kris Verheyen. *Plant functional trait response to environmental drivers across European temperate forest understorey communities.* Accepted at Plant Biology.

Haben Blondeel, Elyn Remy, Michael P. Perring, Samuel Bodé, Dries Landuyt, Pieter De Frenne, Pascal Boeckx, Kris verheyen. *Larger direct than indirect effects of multiple environmental changes on leaf nitrogen of forest herbs* (in press). Plant and Soil

Dries Landuyt, Emiel De Lombaerde, Michael P. Perring, Lionel R. Hertzog, Evy Ampoorter, Sybryn L. Maes, Pieter De Frenne, Shiyu Ma, Willem Proesmans, **Haben Blondeel**, Bram K. Sercu, Bin Wang, Safaa Wasof, Kris Verheyen. *The functional role of temperate forest understorey vegetation in a changing world.* Global Change Biology 25(11), 3625 - 3641

Bin Wang, **Haben Blondeel**, Lander Baeten, Ika Djukic, Emiel De Lombaerde, Kris Verheyen. *The direct effects of human-induced environmental changes on litter decomposition in temperate forest are eclipsed by their indirect effects via the forest understorey vegetation* (in press). Soil Biology and Biogeochemistry

Leen Depauw, Dries Landuyt, Michael P. Perring, **Haben Blondeel,** Sybryn L. Maes, Martin Kopecký, František Máliš, Margot Vanhellemont, Kris Verheyen (in press). *A general framework for quantifying the effects of land-use history on ecosystem dynamics*. Ecological Indicators

Leen Depauw, Michael P. Perring, Jörg Brunet, Sybryn L. Maes, **Haben Blondeel**, Emiel De Lombaerde, Robbe De Groote, Kris Verheyen (in press). *Interactive effects of past land use and recent forest management on the understorey community in temperate oak forests in South Sweden*. Journal of Vegetation Science.

Sybryn L Maes, **Haben Blondeel**, Michael P Perring, Leen Depauw, Guntis Brūmelis, Jörg Brunet, Guillaume Decocq, Jan Den Ouden, Werner Härdtle, Radim Hédl, Thilo Heinken, Steffi Heinrichs, Bogdan Jaroszewicz, Keith Kirby, Martin Kopecký, František Máliš, Monika Wulf, Kris Verheyen (2019). *Litter quality, land-use history, and nitrogen deposition effects on topsoil conditions across European temperate deciduous forests*. Forest Ecology and Management (433); 405 - 418

Haben Blondeel, Michael P. Perring, Laurent Bergès, Jörg Brunet, Guillaume Decocq, Leen Depauw, Martin Diekmann, Dries Landuyt, Jaan Liira, Sybryn L. Maes, Margot Vanhellemont, Monika Wulf, Kris Verheyen (2019). *Context-Dependency of Agricultural Legacies in Temperate Forest Soils*. Ecosystems (22:4); 781 - 795

Michael P. Perring, Martin Diekmann, Gabriele Midolo, David Schellenberger Costa, Markus Bernhardt-Römermann, Johanna C.J. Otto, Frank S. Gilliam, Per-Ola Hedwall, Annika Nordin, Thomas Dirnböck, Samuel M. Simkin, František Máliš, **Haben Blondeel**, Jörg Brunet, Markéta Chudomelová, Tomasz Durak, Pieter De Frenne, Radim Hédl, Martin Kopecký, Dries Landuyt, Daijiang Li, Peter Manning, Petr Petřík, Kamila Reczyńska, Wolfgang Schmidt, Tibor Standovár, Krzysztof Świerkosz, Ondřej Vild, Donald M. Waller, Kris Verheyen (2018). *Understanding context dependency in the response of forest understorey plant communities to nitrogen deposition*. Environmental Pollution (242)

Michael P Perring, Markus Bernhardt-Römermann, Lander Baeten, Gabriele Midolo, **Haben Blondeel,** Leen Depauw, Dries Landuyt, Sybryn L Maes, Emiel De Lombaerde, Maria Mercedes Carón, Mark Vellend, Jörg Brunet, Markéta Chudomelová, Guillaume Decocq, Martin Diekmann, Thomas Dirnböck, Inken Dörfler, Tomasz Durak, Pieter De Frenne, Frank S Gilliam, Radim Hédl, Thilo Heinken, Patrick Hommel, Bogdan Jaroszewicz, Keith J Kirby, Martin Kopecký, Jonathan Lenoir, Daijiang Li, František Máliš, Fraser J G Mitchell, Tobias Naaf, Miles Newman, Petr Petřík, Kamila Reczyńska, Wolfgang Schmidt, Tibor Standovár, Krzysztof Świerkosz, Hans Van Calster, Ondřej Vild, Eva Rosa Wagner, Monika Wulf, Kris Verheyen (2018). *Global environmental change effects on plant community composition trajectories depend upon management legacies*. Global Change Biology (24:4); 1722 - 1740

Pieter De Frenne, **Haben Blondeel**, Jörg Brunet, Maria Mercedes Caron, Olivier Chabrerie, Mathias Cougnon, Sara A.O. Cousins, Guillaume Decocq, Martin Diekmann, Bente J. Graae, Mick E. Hanley, Thilo Heinken, Martin Hermy, Annette Kolb, Jonathan Lenoir, Jaan Liira, Anna Orczewska, Anna Shevtsova, Thomas Vanneste, Kris Verheyen (2018). *Atmospheric nitrogen deposition on petals enhances seed quality of the forest herb Anemone nemorosa*. Plant Biology (20:3); 619 - 626

Kris Verheyen, Pieter De Frenne, Lander Baeten, Donald M. Waller, Radim Hédl, Michael P. Perring, **Haben Blondeel**, Jörg Brunet, Markéta Chudomelová, Guillaume Decocq, Emiel De Lombaerde, Leen Depauw, Thomas Dirnböck, Tomasz Durak, Ove Eriksson, Frank S. Gilliam, Thilo Heinken, Steffi Heinrichs, Martin Hermy, Bogdan Jaroszewicz, Michael A. Jenkins, Sarah E. Johnson, Keith J. Kirby, Martin Kopecký, Dries Landuyt, Jonathan Lenoir, Daijiang Li, Martin Macek, Sybryn L. Maes, František Máliš, Fraser J. G. Mitchell, Tobias Naaf, George Peterken, Petr Petřík, Kamila Reczyńska, David A. Rogers, Fride HØistad Schei, Wolfgang Schmidt, Tibor Standovár, Krzysztof Świerkosz, Karol Ujházy, Hans Van Calster, Mark Vellend, Ondřej Vild, Kerry Woods, Monika Wulf, Markus Bernhardt-

Römermann (2017). *Combining Biodiversity Resurveys across Regions to Advance Global Change Research*. BioScience (67:1); 73 - 83

Pieter Vangansbeke, **Haben Blondeel**, Dries Landuyt, Pieter De Frenne, Leen Gorissen, Kris Verheyen (2017). *Spatially combining wood production and recreation with biodiversity conservation*. Biodiversity and Conservation (26:13); 3213 - 3239

Michael P Perring, Pieter De Frenne, Lander Baeten, Sybryn L Maes, Leen Depauw, **Haben Blondeel**, Maria Mercedes Carón, Kris Verheyen (2016). *Global environmental change effects on ecosystems: The importance of land-use legacies*. Global Change Biology (22:4); 1361 - 1371

Under review

Haben Blondeel, Michael P. Perring, Leen Depauw, Dries Landuyt, Pieter De Frenne, Kris Verheyen. *Light and warming drive forest understorey community development in different envitonments.* Major revisions at Global Change Biology

Haben Blondeel, Michael P. Perring, Emiel De Lombaerde, Leen Depauw, Dries Landuyt, Sanne Govaert, Sybryn L. Maes, Pieter Vangansbeke, Pieter De Frenne, Kris Verheyen. *Individualistic responses of forest herb traits to environmental change.* Resubmitted to Plant Biology

Michael P. Perring, Pieter De Frenne, Lionel R. Hertzog, **Haben Blondeel,** Leen Depauw, Sybryn L. Maes, Safaa Wasof, Hans Verbeeck, Kris Verheyen, and forestREplot. *Resurveys demonstrate an increase in liana frequency in temperate forests across Europe*. Major revisions at Frontiers in Ecology and Evolution.

Dries Landuyt, Sybryn L. Maes, Leen Depauw, Evy Ampoorter., **Haben Blondeel**, Michael P. Perring, Guntis Brümelis, Jörg Brunet, Guillaume Decocq, Werner Härdtle, Radim Hédl, Thilo Heinken, Steffi Heinrichs, Bogdan Jaroszewicz, Keith J Kirby, Martin Kopecky, František Máliš, Monika Wulf, Kris Verheyen. *Drivers of aboveground biomass and nutrient stocks in the understorey of temperate deciduous forests across Europe.* Minor Revisions at Journal of Ecology

Peer reviewed articles in A2 journals

Haben Blondeel, Pieter Vangansbeke (2016). *Ruimtelijke planning van biodiversiteit, houtproductie en recreatie: Drie verschillende houtproductiescenario's geven flexibiliteit in beheer.* Vakblad Natuur, Bos en Landschap (13:126), 16 - 19

Haben Blondeel, Pieter Vangansbeke (2016). *Biodiversiteit, recreatie en biomassaproductie in Vlaamse bossen: hand in hand of neus aan neus?* Bosrevue (55)

Scientific activities

Participation in symposia with oral presentation

Haben Blondeel, Elyn Remy, Michael P. Perring, Dries Landuyt, Samuel Bodé, Pieter De Frenne, Pascal Boeckx, Kris Verheyen. *Functional traits dictate soil nitrogen (N) uptake by plants in response to land-use legacies and environmental change.* British Ecological Society (BES) Annual meeting. 19/12/2018, Birmingham, United Kingdom.

Haben Blondeel. *Land-use legacies affect forest herbs under global change: a large-scaled multi-factor experiment.* European Geosciences Union (EGU) General Assembly. 13/04/2018, Vienna, Austria.

Haben Blondeel, Elyn Remy, Michael P. Perring, Kris verheyen. *Land-use legacies and reactive nitrogen (N) affect forest herbs in a large-scale multi-factor experiment.* Long-Term Ecosystem Research in Europe (eLTER) Annual Conference. 28/11/2017, Malaga, Spain.

Haben Blondeel, Pieter De Frenne, Leen Depauw, Sybryn L. Maes, Emiel de Lombaerde, Dries Landuyt, Michael P Perring, Kris Verheyen. *Disentangling interactive effects of past and present environmental changes on forest herbs in a multi-factor experiment.* Flanders Annual Meeting on Ecology (FAME). 19/12/2016, Ghent, Belgium.

Haben Blondeel, Pieter Vangansbeke, Kris Verheyen. *Biodiversiteit, recreatie en biomassaproductie in Vlaamse bossen: hand in hand of neus aan neus?* Startersdag natuur- en bosonderzoek. 27/03/2015, Brussels, Belgium.

Participation in symposia with poster presentation

Haben Blondeel, Michael P Perring, Elyn Remy, Dries Landuyt, Emiel de Lombaerde, Leen Depauw, Sybryn L. Maes, Kris Verheyen. *Land-use legacies affect forest herbs under global change: A large multi-factor experiment.* Ecology Across Borders (EAB) meeting. 13/12/2017, Ghent, Belgium.

Participation in other scientific events with an active contribution

Sanne Van Den Berge, Jonathan Janssens, Frederick De Roeck, Elvira De Cauter, **Haben Blondeel.** *Grey areas in green revolutions: A transdisciplinary analysis of cobalt extraction (TRACE) for green technologies*. Research Proposal Contest on the Sustainable Development Goals at the UGent Faculty of Bioscience Engineering, in light of the honorary doctorate to prof. Johan Rockström. 22/03/2018, Ghent, Belgium

Maurice Hoffman, Kathy Steppe, Arne Verstraeten, **Haben Blondeel**, Kris Verheyen. *Guided Post-conference Tour: Visit the LTER Site Gontrode.* Ecology Across Borders (EAB) meeting. 13/12/2017, Ghent, Belgium.

Scientific Awards

Toekomstboom (2015). *Beste studentenscriptie in het vakgebied van bosecologie en bosbeheer uit Nederland en Vlaanderen.* Awarded by Stichting Toekomsboom.

Startersdag natuur – en bosonderzoek (2015). *Beste lezing op de startersdag natuur- en bosonderzoek.* Selected by the audience

Tutor of master dissertations

Guiditta Franci (2017-2018). Analisi sperimentale delle risposte della vegetazione erbacea forestale ai principali drivers del cambiamento climatico globale. Firenze University. Promoted by Federico Selvi and Kris Verheyen. Tutored by **Haben Blondeel.**

Adrien Berquer (2016-2017). *Quercus robur*'s phenology and understorey communities: How environmental factors act or interact? Université de Lille & Ghent University, Promoted by Pieter De Frenne, tutored by **Haben Blondeel** and Sumitra Dewan.