Adaptation of Understorey Herbs to Forest Management

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SUMMARY

In our rapidly changing world, land use has been recognized as having one of the strongest impacts on species and genetic diversity. The present state of temperate forests in Europe is a product of decisions made by former and current management and policy actions, rather than natural factors. Alterations of crown projection areas, structural complexity of the forest stand caused by thinning and cuttings, and changes in tree species composition caused by regeneration or plantings not only affect forest interior buffering against warming, but also the understorey light environment and nutrient availability. Ultimately, current silvicultural management practices have deep impact on the forest ecosystems, microenvironmental changes and forest floor understorey herbs. In response to environmental changes, plants rely on genetically heritable phenotypic variation, an important level of variation in the population, as it is prerequisite for adaptation. However, until now most studies on plant adaptation to land use focus on grassland management. Yet, studies on the adaptation of forest understorey herbs to forest management have been absent so far. This is important because understanding adaptation of understorey herbs is crucial for biodiversity conservation, forest restoration, and climate change mitigation. Studying current adaptation of understorey herbs to forest management yields insights into the evolutionary consequences of management practices, which could be employed to improve sustainable use of forest habitat.

To understand adaptation of understorey herbs to forest management, I investigated populations of five different forest understorey herb species in a series of experiments. More precisely, I investigated the genetic differentiation among populations, whether phenotypic trait variation correlate with forest structural attributes and microenvironmental variables, and if forest structure has a direct effect on genetically based phenotypic variation, or indirect effects through the microenvironment (**Chapter 1**). Furthermore, I investigated the presence of local adaptation of the understorey herbs, *Anemone nemorosa* and *Milium effusum*, to forest management and forest structural attributes, and tested dispersal versus establishment limitations (**Chapter 2**). Lastly, I covered the newly emerged ecological topic of intra-individual variation, using the forest herb *Galium odoratum* as a study species. I compared trait variation within various hierarchical levels of biological organisation, tested the genetic basis of intra-individual trait variation along with the effects of forest management and related microenvironmental factors, and investigated

the effects of experimentally induced drought and earlier shading in plastic responses and trait means (**Chapter 3**).

For **Chapter 1**, I sampled individuals of five forest understorey herbs (*Anemone nemorosa, Galium odoratum*, *Milium effusum*, *Oxalis acetosella*, and *Viola reichenbachiana*) wherever they were present from 150 forest plots covering a broad management intensity gradient. I then transplanted them into pots and cultivated them under common garden conditions. For two growing seasons I measured flowering start and end, plant height, number of ramets and flowering ramets, on all study species. Since any observed trait differences among populations under a common garden setting can be ascribed to a genetic basis, results from **Chapter 1** showed that forest understorey herbs varied genetically in the measured functional and phenological traits among the sampled populations. Structural equation models revealed that forest management affected the traits either directly through variation in forest structural attributes or indirectly through changes in the microclimatic environment. However, the directions and strength of the effects varied depending on the study species, and some species were not affected by the investigated variables at all. Ultimately, forest management was shown to have potential evolutionary consequences for forest understorey herbs. Based on these results, I recommend diverse forest management actions within landscapes as it creates heterogeneity that selects for different plant traits and thus helps conserving genetic diversity.

For **Chapter 2**, I conducted a reciprocal transplant experiment along a forest management intensity gradient. Working on a subset of plots, I transplanted individuals of *A. nemorosa* and *M. effusum* ‘sympatrically’, ‘near allopatrically’, ‘far allopatrically’ and to ‘absent sites’ where the species did not naturally occur. My results from **Chapter 2** showed that these two forest understorey herbs, possessing contrasting dispersal abilities and gene flow, vary in the amount of phenotypic plasticity and local adaptation to forest management and microenvironment. Whereas *A. nemorosa* always performed better at home sites compared to sites where the species did not naturally occur, I found the opposite pattern for *M. effusum*. Furthermore, *A. nemorosa* was locally adapted to structural complexity of the forest stands. However, majority of the measured traits elicited plastic responses when transplanted allopatrically, indicating that forest understorey herbs generally rely more heavily on phenotypic plasticity for producing appropriate plant trait responses to foreign forest management and microenvironmental changes.

For **Chapter 3**, I conducted another common garden experiment. I sampled genets of *G.* odoratum from a subset of plots covering the management intensity gradient within each region. I propagated each genet into four ramets, and applied one of the four treatments: control, early shading, drought, or combined treatments to each ramet in a full factorial design. I measured plant height, leaf length, and leaf width at all hierarchical levels of organisation to calculate the inter- and intra-individual variation (CV). Results from **Chapter 3** showed that the majority of the total variation was located at the lowest hierarchical levels intra-shoot and intra-ramet, and significant variation in CV of plant height and leaf width among populations of origin indicates that these two traits are partly genetically based. I observed correlations between soil temperature and CV in plant height indicating adaptation at local conditions. Furthermore, the applied drought treatment reduced intra-shoot CV in leaf width, while early shading treatment increased intra-ramet CV in leaf length. Finally, by correlating CV and trait means, I found that the mean and CV of the intra-shoot leaf width were independent under control conditions but correlated under drought, indicating that the two components are interdependent and that one could drive the other. Ultimately, my results indicate that intra-individual variation can evolve and may be adaptive.

Some methodological limits and potential future perspectives should be highlighted. I could have improved **Chapter 1** by matching more of the measured traits among species. Traits such as emergence and proportion of flowering ramets were species specific, and I had to apply trait proxies to enable cross-species comparisons. To increase the robustness of the results, future studies should aim for a greater overlap of the measured traits that have a clear link to population dynamics and performance in all the included study species. Furthermore, not all species were affected by the chosen forest structural attributes and environmental variables. Future studies could include other important forest structural attributes, such as tree basal area, stem density or canopy closure. Furthermore, more rigorously investigation of each species by including more measured traits might have revealed additional significant pattern. However, there is also a chance that the lack of effects in some species is simply due to neutral variation with respect to any environmental variables. **Chapter 2** could have greatly benefitted if I could have had a longer experimental duration of the reciprocal transplant, allowing for measurements for over more than two growing seasons in the investigated perennial species. The first year after the transplant yielded no significant results and should rather be viewed as an establishment phase. The second year revealed local adaptation and phenotypic plasticity, but it would be valuable to see whether these results persist in the following years. Furthermore, I encourage future studies to include more species covering the dispersal rate and gene flow gradient, allowing to link life-history strategies to the strength of local adaptation. Due to a combination of transplant shock and unfavourable climatic conditions in the establishment phase, **Chapter 3** suffered great mortality at the start of the experiment. Greater care should be taken in the future to protect experimental pots from unexpected late frosts. Furthermore, future experiments should include sexual reproductive traits, which remained an unexplored area here, as comparisons of intra-individual variation in sexual *versus* reproductive traits within the same individuals remains a research gap.

In sum, my conducted experiments complement each other well and managed to fill in research gaps on the topic of genetically heritable phenotypic variation in understorey herbs and how it is affected by forest management and related microenvironmental variables. I showed that forest management has direct evolutionary consequences on the genetic basis of understorey herbs, but also indirectly through the microenvironment. Furthermore, I revealed that local adaptation and phenotypic plasticity of understorey herbs to forest structural attributes act along continuous gradients. And lastly, I highlighted the important role of intra-individual variation by revealing plastic responses to drought and shading, urging researchers to not ignore this important level of trait variation. Ultimately, understorey herbs in temperate forests employ phenotypic plasticity as a flexible strategy to adapt to varying environmental conditions. By adjusting their leaf characteristics, reproductive investment, and phenology, they can optimize their fitness and survival in response to changes in light availability, resource availability, and seasonal cues. The anthropogenic impact on temperate forests and understorey herbs will continue and likely increase in the future. This should urge foresters to adapt their silvicultural management decisions towards the long-term preservation of genetic diversity and, through this, the evolvability and adaptability of forest understorey herbs and associated organisms. Based on the results shown in my dissertation, variation in forest management regimes and types could be beneficial for promoting genetic diversity within several species of forest understorey herbs. Lastly, in the face of future climatic changes, the mechanisms by which plants can cope with increasing stressful environmental conditions might very well rely heavily on intra-individual variation, providing the necessary rapid plastic adjustment to changing microclimatic conditions within populations and thus increase climate change resilience.

ZUSAMMENFASSUNG

In unserer sich rapide verändernden Welt ist die Landnutzung als einer der stärksten Einflüsse auf Arten- und genetische Vielfalt anerkannt. Der gegenwärtige Zustand der gemäßigten Wälder in Europa ist das Ergebnis von Entscheidungen früherer und aktueller Bewirtschaftung und politischer Maßnahmen, und nicht natürlicher Faktoren. Veränderungen in der Kronendichte und strukturellen Komplexität des Waldbestandes durch Auflichtung und Holzeinschlag sowie Veränderungen der Baumarten-zusammensetzung durch Naturverjüngung oder Pflanzungen beeinflussen nicht nur das Waldinnenraumklima, sondern auch die Lichtverhältnisse und Nährstoffverfügbarkeit im Unterwuchs. Die derzeitigen forstwirtschaftlichen Praktiken haben somit eine tiefgreifende Auswirkung auf die Waldökosysteme, die unmittelbare Umwelt und den Bodenbewuchs. Als Reaktion auf Umweltveränderungen sind Pflanzen auf genetisch vererbbare phänotypische Variation angewiesen, die eine wichtige Ebene der Variation in der Population darstellt und eine Voraussetzung für Anpassungsfähigkeit ist. Bisher konzentrieren sich die meisten Studien zur Anpassung von Pflanzen an die Landnutzung auf Grünlandbewirtschaftung, während Studien zur Anpassung von Pflanzen der Krautschicht an die Waldbewirtschaftung fehlen. Diese sind jedoch wichtig, da das Verständnis der Anpassungsfähigkeit der Krautschicht im Wald für den Erhalt der Biodiversität, die ökologische Verbesserung der Waldstruktur der Wälder und die Minderung des Klimawandels von entscheidender Bedeutung ist. Die Untersuchung der aktuellen Anpassung von Pflanzen der Krautschicht an die Waldbewirtschaftung liefert Erkenntnisse über die evolutionären Folgen der Bewirtschaftungspraktiken, die zur Verbesserung der nachhaltigen Nutzung von Wäldern genutzt werden könnten.

Um die Anpassungsfähigkeit der Krautschicht an die Waldbewirtschaftung zu verstehen, habe ich in einer Reihe von Experimenten Populationen fünf verschiedener Arten der Krautschicht untersucht. Genauer gesagt habe ich die genetische Differenzierung der Populationen untersucht und ob phänotypische Merkmalsvariationen mit der Waldstruktur und Umweltvariablen korrelieren, Außerdem habe ich untersucht ob die Waldstruktur einen direkten Einfluss auf genetisch bedingte phänotypische Variation hat oder indirekte Effekte über die unmittelbare Umgebung vermittelt (**Kapitel 1**). Darüber hinaus habe ich die lokale Anpassung der Unterwuchskräuter *Anemone nemorosa* und *Milium effusum* an die Waldbewirtschaftung und die waldbaulichen Merkmale untersucht und die

Begrenzungen der Ausbreitung im Vergleich zur Ansiedlung getestet (**Kapitel 2**). Schließlich habe ich mich mit dem neu aufgekommenen ökologischen Thema der intra-individuellen Variation beschäftigt und dabei die Waldpflanze *Galium odoratum* als Untersuchungsart verwendet. Ich habe die Merkmalsvariation in verschiedenen verwandtschaftlichen Ebenen verglichen, die genetische Grundlage der intra-individuellen Merkmalsvariation sowie die Auswirkungen von Waldbewirtschaftung und damit einhergehenden Umfeldveränderungen untersucht und die Effekte von experimentell induzierter Trockenheit und früher Schattierung auf plastische Reaktionen und Merkmalsmittelwerte untersucht (**Kapitel 3**).

Für Kapitel 1 habe ich Individuen von fünf Arten der Krautschicht *(Anemone nemorosa, Galium odoratum, Milium effusum, Oxalis acetosella* und *Viola reichenbachiana*) an 150 Waldstandorten entlang eines breiten Bewirtschaftungsgradienten gesammelt, sie in Töpfe umgepflanzt und unter sogenannten *common garden* Bedingungen kultiviert. Über zwei Vegetationsperioden hinweg habe ich den Beginn und das Ende der Blütezeit, die Pflanzenhöhe, die Anzahl der Ausläufer und blühenden Ausläufer bei allen untersuchten Arten gemessen. Da jede beobachtete Merkmalsdifferenz zwischen Populationen unter „Common Garden“ Bedingungen auf eine genetische Veränderung zurückzuführen ist, zeigten die Ergebnisse aus **Kapitel 1**, dass sich die Populationen genetisch in den gemessenen funktionellen und phänologischen Merkmalen unterschieden. Strukturgleichungsmodelle zeigten, dass die Waldbewirtschaftung die Merkmale entweder direkt durch Variationen in der Waldstruktur oder indirekt durch Veränderungen der unmittelbaren Umgebung beeinflusste. Die Auswirkungen variierten jedoch je nach untersuchter Art in Richtung und Stärke und einige Arten wurden von den untersuchten Variablen überhaupt nicht beeinflusst. Letztendlich wurde gezeigt, dass die Waldbewirtschaftung potenzielle evolutionäre Konsequenzen für die Krautschicht im Wald hat. Basierend auf diesen Ergebnissen empfehle ich eine vielfältige Bewirtschaftung der Wälder, da dies Heterogenität schafft, die verschiedene Pflanzeneigenschaften fördert und somit zur Erhaltung der genetischen Vielfalt beiträgt.

Für **Kapitel 2** habe ich ein reziprokes Transplantationsexperiment entlang eines Bewirtschaftungsgradienten im Wald durchgeführt. In einem Teil der Untersuchungsflächen habe ich Individuen von *A. nemorosa* und *M. effusum* sowohl ‚sympatrisch‘, ‚nahe allopatrisch‘, ‚weit entfernt allopatrisch‘ als auch an Standorten

‚ohne natürliches Vorkommen' der Arten umgepflanzt. Meine Ergebnisse aus **Kapitel 2** zeigen, dass diese beiden krautigen Pflanzen im Wald, die unterschiedliche Ausbreitungsfähigkeiten und Genflüsse aufweisen, hinsichtlich des Ausmaßes der phänotypischen Plastizität und lokalen Anpassung an die Waldbewirtschaftung und die unmittelbare Umgebung variieren. Während *A. nemorosa* immer besser an den Heimatstandorten abschnitt im Vergleich zu Standorten, an denen die Art nicht natürlicherweise vorkam, ergab sich für *M. effusum* das umgekehrte Muster. Darüber hinaus war *A. nemorosa* an die strukturelle Komplexität der Waldbestände lokal angepasst. Jedoch zeigten die meisten gemessenen Merkmale plastische Reaktionen, wenn sie allopatrisch umgepflanzt wurden, was darauf hinweist, dass die Krautschicht im Wald im Allgemeinen stärker auf phänotypische Plastizität angewiesen ist, um geeignete Eigenschaften als Reaktion auf neue Waldbewirtschaftung und unmittelbare Umweltveränderungen zu entwickeln.

Für **Kapitel 3** habe ich ein weiteres *common garden* Experiment durchgeführt. Ich habe Geneten von *G. odoratum* von einem Teil der Flächen entlang des Bewirtschaftungsgradienten in jeder Region entnommen. Jeder Genet wurde in vier Rameten unterteilt, welche einer der vier Behandlungen – ‚Kontrolle', ‚früher Schatten', ‚Trockenheit' oder ‚kombinierte Behandlungen' - gemäß eines vollständig faktoriellen Designs unterzogen wurden. Ich habe Pflanzenhöhe, Blattlänge und Blattbreite auf allen verwandtschaftlichen Ebenen gemessen, um die inter- und intra-individuelle Variation zu berechnen. Die Ergebnisse aus **Kapitel 3** zeigten, dass der Großteil der Gesamtvariation auf den niedrigsten hierarchischen Ebenen innerhalb der Sprosse und innerhalb der Rameten zu finden war. Signifikante Variationen im Variationskoeffizienten (CV) der Pflanzenhöhe und Blattbreite zwischen den Ursprungspopulationen deuten darauf hin, dass diese beiden Merkmale zum Teil genetisch bedingt sind. Ich beobachtete Korrelationen zwischen der Bodentemperatur und dem CV der Pflanzenhöhe, was auf eine Anpassung an lokale Bedingungen hindeutet. Darüber hinaus reduzierte die angewendete Trockenbehandlung den intra-spross CV in der Blattbreite, während die frühe Beschattung den intra-ramet CV in der Blattlänge erhöhte. Schließlich stellte ich durch die Korrelation von CV und Merkmalsmittelwerten fest, dass der Mittelwert und der CV der intra-spross Blattbreite unter Kontrollbedingungen unabhängig voneinander waren, aber unter Trockenheit korrelierten, was darauf hindeutet, dass die beiden Komponenten voneinander abhängig sind und dass eine die andere beeinflussen kann. Letztendlich deuten meine

Ergebnisse darauf hin, dass die intra-individuelle Variation sich entwickeln kann und möglicherweise anpassungsfähig ist.

Einige methodische Einschränkungen und potenzielle zukünftige Perspektiven sollten hervorgehoben werden. Das in **Kapitel 1** beschriebene Experiment hätte verbessert werden können, indem mehr der gemessenen Merkmale zwischen den Arten abgeglichen worden wären. Merkmale wie das Auftreten und der Anteil blühender Sprossen waren artspezifisch und ich musste Merkmalsproxys verwenden, um Vergleiche zwischen den Arten zu ermöglichen. Um die Robustheit der Ergebnisse zu erhöhen, sollten zukünftige Studien darauf abzielen, eine größere Überschneidung der gemessenen Merkmale zu erreichen, die einen klaren Zusammenhang mit der Populationsdynamik und Leistung in allen untersuchten Arten aufweisen. Darüber hinaus waren nicht alle Arten von den gewählten strukturellen Waldeigenschaften und Umweltvariablen betroffen. Zukünftige Studien könnten andere wichtige strukturelle Waldeigenschaften wie den Brusthöhendurchmesser der Bäume, die Stammdichte oder den Kronenschluss einbeziehen. Zudem könnte eine gründlichere Untersuchung jeder Art durch den Einsatz weiterer gemessener Merkmale möglicherweise signifikantere Muster aufzeigen. Es besteht jedoch auch die Möglichkeit, dass das Fehlen von Effekten bei einigen Arten einfach auf neutrale Variation im Hinblick auf Umweltvariablen zurückzuführen ist. **Kapitel 2** hätte erheblich von einer längeren experimentellen Dauer des reziproken Transplantationsexperiments profitiert, um Messungen über mehr als zwei Vegetationsperioden bei den untersuchten mehrjährigen Arten zu ermöglichen. Das erste Jahr nach dem Transplantieren ergab keine signifikanten Ergebnisse und sollte eher als Etablierungsphase betrachtet werden. Das zweite Jahr zeigte lokale Anpassung und phänotypische Plastizität, aber es wäre wichtig zu sehen, ob diese Ergebnisse in den folgenden Jahren Bestand haben. Darüber hinaus ermutige ich zukünftige Studien, mehr Arten einzubeziehen, die den Gradienten der Ausbreitungsrate und des Genaustauschs abdecken, um eine Verbindung zwischen Strategien der verschiedener Lebensstadien und der Stärke der lokalen Anpassung herzustellen. Aufgrund einer Kombination aus Transplantationsschock und ungünstigen klimatischen Bedingungen in der Etablierungsphase konnte in **Kapitel 3** zu Beginn des Experiments eine hohe Sterblichkeitsrate beobachtet werden. In Zukunft sollte größere Sorgfalt darauf verwendet werden, die Pflanztöpfe vor Spätfrost zu schützen. Darüber hinaus sollten zukünftige Experimente sexuelle Reproduktionsmerkmale einbeziehen, da dieser Bereich hier unerforscht blieb. Der

Vergleich der intra-individuellen Variation sexueller und reproduktiver Merkmale innerhalb desselben Individuums stellt ebenfalls eine Forschungslücke dar.

Zusammenfassend ergänzen sich meine durchgeführten Experimente gut. Forschungslücken zum Thema der Auswirkungen der Waldbewirtschaftung und damit einhergehenden Umweltvariablen auf die phänotypische Variation der Krautschicht und deren Vererbung konnten geschlossen werden. Ich habe gezeigt, dass die Waldbewirtschaftung direkte evolutionäre Auswirkungen auf die genetische Basis von Pflanzen der Krautschicht hat, aber auch indirekt über die nahe Umgebung. Darüber hinaus habe ich gezeigt, dass lokale Anpassung und phänotypische Plastizität von krautigen Pflanzen an Waldstrukturen entlang kontinuierlicher Gradienten wirken. Schließlich habe ich die wichtige Rolle der intra-individuellen Variation hervorgehoben, indem ich plastische Reaktionen auf Trockenheit und Beschattung zeigen konnte, welche als wichtige Ebene der Merkmalsvariation in Folgestudien nicht vernachlässigt werden sollte. Letztendlich setzen Unterwuchskräuter in gemäßigten Wäldern phänotypische Plastizität als flexible Strategie ein, um sich an sich verändernde Umweltbedingungen anzupassen. Durch Anpassung ihrer Blatteigenschaften, ihres Reproduktionsaufwands und ihrer Phänologie können sie ihre Fitness optimieren und ihre Überlebenschancen steigern, indem sie auf Veränderungen in der Lichtverfügbarkeit, der Ressourcenverfügbarkeit und saisonalen Signalen reagieren. Der anthropogene Einfluss auf gemäßigte Wälder und ihre Krautschicht wird auch in Zukunft anhalten und voraussichtlich zunehmen. Daher sollten Forstleute ihre waldbaulichen Entscheidungen und Maßnahmen anpassen, um die langfristige Erhaltung der genetischen Vielfalt und damit die Entwicklungsfähigkeit und Anpassungsfähigkeit von krautigen Pflanzen und den damit verbundenen Organismen sicherzustellen. Basierend auf den in meiner Dissertation gezeigten Ergebnissen ist die Vielfalt der Waldbewirtschaftungsmaßnahmen wahrscheinlich förderlich für die genetischen Vielfalt innerhalb mehrerer Arten von krautigen Pflanzen. Schließlich könnte angesichts zukünftiger klimatischer Veränderungen der Mechanismus, mit dem Pflanzen mit zunehmend stressigen Umweltbedingungen umgehen können, stark von der intra-individuellen Variation abhängen, um notwendige schnelle plastische Anpassungen an sich verändernde Umweltbedingungen innerhalb von Populationen zu ermöglichen und so die Klimaresilienz zu erhöhen.

GENERAL INTRODUCTION

My thesis deals with the overarching question whether understorey herbs can be adapted to management of European temperate forests. Before diving into the scope and the aims of my dissertation, I here first give a general introduction to temperate forests, how they are managed, the associated microenvironment, global change, and

Box 1

Fitness is known as the reproductive success and output of an individual. Individuals that survive and produce more offspring than others are said to have a higher fitness (Conner & Hartl, 2004; Silvertown & Charlesworth, 2009). We often use various vegetative traits such as plant height and biomass as fitness proxies, even though they are not necessarily a direct measure of reproductive success, such as number of offspring or number of seeds produced. In plants, especially in clonal plants, fitness can be studied on many different hierarchical levels of biological organisation. One **genet** (a genetically identical individual) can produce several **ramets** (potentially independent individuals derived vegetatively from a single parent, e.g., via rhizomes). However, many factors come into play and contributes either positively or negatively to an individual's fitness.

Genetic variation is the variation of allele presentation at a given locus within a population, whereas **genetic differentiation** is the variation of allele presentation at a given locus among populations. Genetic variation is ubiquitous in natural populations, but not for all loci or traits. Genetic variation can be influenced by mutation, genetic drift, gene flow, and natural selection (Conner & Hartl, 2004). A **mutation** is a change in the nucleic acid sequence of an individual. **Gene flow** is the changes in gene frequency brought about by the migration of new individuals into a population (Silvertown & Charlesworth, 2009), either by seed, pollen, rhizomes or stolons. **Genetic drift** is the change in the frequency of an existing gene variation in a population due to random chance. **Natural selection** is the process resulting in adaptive evolutionary change, where a certain phenotype in a population with a higher fitness is selected for. Natural selection can take place when there is variation between individuals in heritable traits (Silvertown & Charlesworth, 2009), or between different non-heritable phenotypes that environmental selection acts on.

A **phenotype** of an individual is expressed depending on its genetic constitution (i.e., **genotype**) and the environment the individual is exposed to. The variation in an individual's phenotype when exposed to different environments is called **phenotypic plasticity** (Silvertown & Charlesworth, 2009). Phenotypic plasticity can occur in various traits and the breadth of phenotypic plasticity can have a heritable component, that may be selected for independently of the mean trait value (Silvertown & Charlesworth, 2009). As a result of genotypic variation and phenotypic plasticity, we have **intraspecific trait variation**. Intraspecific trait variation can be influenced by abiotic and biotic cues that induce phenotypic plasticity (Westerband *et al.*, 2021). In plants, we can also study the **intra-individual variation**, and in clonal plants, across several hierarchical levels of biological organisation (i.e., intra-shoot, intra-clone, intra-ramet). Lastly, **local adaptation** is a result of divergent selection and evolution, equipping local populations with a fitness advantage under its local environmental conditions compared to foreign environments (Kawecki & Ebert, 2004).

forest understorey herbs. A short introduction to evolutionary ecology and the key terms used within this field can be found in **Box 1**.

TEMPERATE FORESTS

Temperate forest ecosystems cover a large climatic range and typically consist of several tree species (Spiecker, 2003), with the top dominant growth stock species in Europe being *Pinus sylvestris*, *Picea abies*, *Fagus sylvatica*, and *Quercus robur* (Wolfslehner *et al*., 2020). European temperate forests provide many important services such as timber production, recreational space, water conservation, and even erosion control (Spiecker, 2003). The majority of German forests are managed as even-aged ‘high forest’, but in fact, Germany holds one of the higher proportions of uneven-aged forests at around 15% of its total forests (Spiecker, 2003). Given the wide range of management regimes commonly practiced, German forests are relevant to include in scientific studies.

Forest management

In our rapidly changing world, land use has been recognized as having the strongest impact on species and genetic diversity (IPCC, 2014). The present state of most temperate forests in Europe is a product of decisions made by former and current management rather than natural factors (Spiecker, 2003). Former management decisions last through time and become land use legacies (Depauw *et al.*, 2019), continuously affecting forest ecosystems as we experience them today. For example, in an effort to counter-act a previous wood shortage (Augusto *et al.*, 2003), coniferous plantations were often favoured due to their easy establishment and quick turn-over time. Coniferous species now appear far beyond their natural range and make up more than 30% of the total forest cover in Germany (Spiecker, 2003). However, we have since learned that tree species composition can have cascading effects on the microclimate of the forest floor (Spiecker, 2003). Current silvicultural management also has great impact on the forest ecosystem. Management decisions impact forest structures, e.g., altering crown projection areas or the structural complexity of the forest. The disturbance to an individual or several tree’s canopies, can alter the physical environment and the microclimate of the forest floor (Gilliam, 2007). Interactions between forest structural attributes and microclimate are interactive and even retroactive: changes in one component will affect and cause adjustment of another (Aussenac, 2000).

Figure 1. Illustrations of two different forest types typically occurring in German temperate forests. Left: Homogenous, age-class coniferous forest. Right: Heterogenous, deciduous forest that can either be selection forest with deadwood retention or an unmanaged forest.

The most common type of forest management is age-class forestry, in which a cohort of trees have all been planted in the same year, and are harvested at once, after which the cycle repeats (Figure 1). Age-class forests are monospecific and mainly consist of coniferous or broad-leaved species with a quick turnover time, e.g. spruce and poplar (Messenger, 1980; Ranger & Claude, 1992; Augusto *et al.*, 2015). Another management type is selection forest. Here, selected trees, usually large individuals, are selected for cutting and subsequently transported out of the forest (Figure 1). The disturbance levels in a selection forest are low but continuous in comparison to age-class forests. Lastly, there is also the occurrence of protected or unmanaged forests, where no trees are cut and no deadwood removed.

Forest structural attributes

Forest management activities affect many different forest structural attributes, which in turn affect the microenvironment. Depending on the forest management type, even the choice of tree species based on their shade casting ability, the tree regeneration type, rotation length of the forest stands, control of large herbivores, and the presence of a shrub layer, can all affect the microclimate of the forest (De Frenne *et al.*, 2021). Throughout my dissertation, I will focus more on some key forest structural attributes than others, and I will now shortly explain them.

The type of tree species growing in a forest can be the results of an active management decision based on either demand or local conditions (e.g., soil pH). In return, the tree species can also influence the local soil properties. Tree species have also been found to influence the understorey vegetation (Augusto *et al.*, 2003), and the **percentage of conifers** in a given forest plot is often used as an important trait to describe forests. Furthermore, **crown projection area** (m^2/ha) is highly affected by the types of tree species growing in the forest. Big deciduous trees such as beech have big tree crowns, which can even create a "forest roof", resulting in a buffering effect against extreme temperatures inside the forest interior (De Frenne *et al*., 2021). Larger crown projection areas have also been found in forest stands that are more homogenous and less structurally complex. Similar variables often used include basal area, stem density, and canopy closure. **Structural complexity index** is used to quantify the structural complexity of the forest stand based on the horizontal and vertical profiles of the forest (Ehbrecht *et al.*, 2017). Complex forest stands are more heterogenous and provide support to ecosystem stability, resilience, and biodiversity (Campbell *et al.*, 2009; Dolezal *et al.*, 2020). To capture disturbance levels caused by forest management, an index for forest management intensity (silvicultural management intensity, **SMI**) has been developed by Schall and Ammer (2013). This complex index is a function of tree species, stand age, and living and dead wooden biomass (Schall & Ammer, 2013). Applying the SMI index to German temperate forest will often rank tree species accordingly from lowest to highest management intensity: oak<beech<pine<<spruce, as coniferous tree species grow faster and have a quicker turnover rate and therefore requires more frequent management.

The forest microenvironment

The microenvironment encompasses biotic and abiotic factors, both above- and below-ground, on a small spatial scale (0.01 to 1000 m; Oke, 1978). The most common and often considered microenvironmental variables include soil and air temperature, precipitation, wind speed, light availability, soil moisture, soil pH, and soil nutrient composition (Davis-Colley *et al.,* 2000). The microenvironment of the forest floor can be highly heterogeneous and is dictated by the forest management regime and forest structural attributes, as the influence of forest management on the vegetation can have implications on the vertical microclimate profile (Onaindia *et al.*, 2004). Age-class forests cause strong environmental impact on the ecosystem during harvest, whereas the abiotic and biotic environment only gradually changes during the growing phase. On the contrary, selection forests are heterogenous in tree demography and size and create varied light conditions, since the areas around cut trees allow sunlight to penetrate down to the forest floor. Moreover, more structurally complex forests also tend to have more heterogeneous microclimates at fine spatiotemporal scales (De Frenne *et al.*, 2021).

Light availability is highly dependent on the tree species, which in turn depend on site-specific climatic factors such as water availability (Aussenac, 2000). Thicker tree canopy and larger crown projection area creates shade, buffers against warming and reduces temperatures inside the forests (De Frenne *et al.*, 2013). Furthermore, shade can limit photosynthesis, or indirectly affect carbon assimilation through morphological and physiological responses (Valladares *et al.*, 2016). However, radiation transmittance does not vary among tree species, but is more dependent on the stand density (Augusto *et al.*, 2003).

Temperatures of the forest floor are also strongly dictated by the forest structural attributes. Forest canopies can act as a buffer of extremities of temperature and humidity variations (De Frenne *et al.*, 2021). The interior of a forest tends to be several degrees colder than in more open habitats (De Frenne *et al.*, 2013). However, selection forests, often supporting coppicing and selective tree extractions, can lead to canopy openings, potentially causing warming of the forest floor with cascading consequences for the composition of understorey herbs (De Frenne *et al.*, 2013). Additionally, the stand species of the forest also indirectly affects temperatures through blocking of the sunlight and radiation transmittance, and the understorey microclimate of coniferous forests are typically colder than that of other forest types (Augusto *et al.*, 2003).

Soil properties are also under strong influence by forest management regimes. Canopy openings caused by tree felling can lead to nutrient depletion of the soil, increasing nitrogen release in the soil, and a decreasing biomass pool (De Frenne *et al.*, 2013). Microclimate also strongly affects soil decomposition via its influence on soil dynamics. Soil pH can be influenced by tree species, and it is often observed that soils are more acidic around coniferous trees (Augusto *et al.*, 2003).

Global change impact

Global change is by now a well-understood phenomenon, it encompasses several human-induced factors, such as climate warming, invasive species, altered precipitation, nutrient fertilization, carbon-dioxide enrichment, and atmospheric nitrogen deposition, all posing serious threats to the biodiversity on our planet (Sala *et al.,* 2000; Zhou *et al.,* 2020). Additionally, global climate change is resulting in **more frequent droughts** (Dai, 2013; Büntgen *et al.*, 2021), also in temperate forests (Trenberth *et al.*, 2014; Millar & Stephenson, 2015; Dai *et al.*, 2018). On top of that, increasing land use is another anthropogenic-induced disturbance that plants must cope with. Ultimately, global change might have various effects and consequences for the microenvironment, as well as direct and indirect effects for understorey herbs.

Due to the buffering effects of the forests, increasing macroclimatic temperatures might be less extreme in the forest interior, and forest understorey herbs have more time for adaptation and migration (Zellweger *et al.*, 2020). This way, temperate forests could potentially be viewed as microrefugia, sustaining a favourable microclimate, which enables species to persist (Gavin *et al.*, 2014). However, as temperatures increase, two-weeks **earlier bud burst and leaf flush** of trees have been observed in Concord, North America since the 1850s, impacting the seasonal forest microclimate. Forest understorey herbs do not match this phenological shift resulting in a short time period of high light to finish their reproductive cycle, and creating a phenological mismatch (Heberling *et al.*, 2019).

As more frequent and severe droughts are expected in the near future, it is therefore essential to investigate how resident species of the forest are adapted to the microenvironment and if they have the genetic basis and phenotypic plasticity required to overcome the obstacles associated with global change.

FOREST UNDERSTOREY HERBS

At the lowest forest strata (<1 meter from the ground), we find the understorey (Gilliam, 2007). The understorey consists of many different species spanning different plant growth forms, from tree seedlings to mosses (Gilliam, 2007). However, in this dissertation I will focus on the herbaceous understorey species, more specifically **graminoids and forbs**. Forest understorey herbs play a key role in the forest ecosystem (Augusto *et al.*, 2003; Gilliam, 2007; Landuyt *et al.*, 2019), harbouring the highest species diversity among all strata in temperate forests (Gilliam, 2007). Besides making up a major part of forest biodiversity, forest understorey herbs also provide important ecosystem services, i.e., forest regeneration (George & Bazzaz, 1999), water cycling (Thrippleton *et al.*, 2018), and nutrient dynamics (Muller, 1978). As the “resident species” of the lower forest strata, forest understorey herbs are under heavy constraints. Besides strong effects from global change, increasing evidence is showing that land use leaves long-term imprints on the composition of forest understorey herbs and its functional diversity (Landuyt *et al.*, 2019).

Study species

I used five perennial forest understorey herbs as study species in this dissertation. They all possess various colonization rates (Brunet *et al.*, 2012), can spread vegetatively to different extents, and have a relatively common occurrence throughout temperate forests in Germany (Martin & Bodson, 2010; Yousefi *et al.*, 2012; Frenne *et al.*, 2017; Migdałek *et al.*, 2017).

Anemone nemorosa

Anemone nemorosa (L. Ranunculaceae), also known as Wood Anemone, is a spring geophyte, reaching a height of 10-25 cm and flowering in April-May (Frederiksen & Rasmussen, 2006). Sexual reproduction is considered to be important for population persistence, seeds are dispersed with gravity and myrmecochory, but vegetative spread through rhizomes is common (Brunet & Oheimb, 1998). *Anemone nemorosa* can produce both reproductive ramets with leaves in whorls of three about two-thirds of the way up the flowering stem, or long-stalked vegetative ramets arising from the rhizome (Shirreffs, 1985a). *Anemone nemorosa* is a shade tolerant species, mostly growing at shaded sites and its yearly cycle is suited to woodland conditions (Shirreffs, 1985a).

Figure 2. *Anemone nemorosa.*

Galium odoratum

Figure 3. *Galium odoratum*

Galium odoratum (L.) Scop. (Rubiaceae), also known as Woodruff, is a forb, reaching heights of 10-30 cm, and flowering in April-June. Reproduction is either sexually via seeds, but also heavily relies on vegetative spread through stolons (Frederiksen & Rasmussen, 2006). Their leaves are lancet-shaped, widest at the middle or just above, and mostly appearing eight at a time, arranged in whorls. They occur primarily in shaded woodlands with moist and rich soils (Frederiksen & Rasmussen, 2006).

Milium effusum

Milium effusum (L., Poaceae), also known as Wood Millet, is a grass, reaching heights of 50-150 cm and flowering in July (Frederiksen & Rasmussen, 2006). Seeds are wind-dispersed, and re-sprouting through short stolons is also used for colonization (De Frenne *et al.*, 2011b). Stems ascends from decumbent bases, where also the flat, smooth, hairless blades are evenly distributed from (Frenne *et al.*, 2017). *Milium effusum* is a shade-tolerant species, growing mostly in temperate deciduous woodland, but can also occur in meadows, along railways and roads, and on rocks (Frenne *et al.*, 2017).

Figure 4. *Milium effusum.*

Oxalis acetosella

Figure 5. *Oxalis acetosella.*

Oxalis acetosella (L., Oxalidaceae), also known as Common Wood Sorrel, is a cleistogamous forb, reaching heights of 5-10 cm and flowering in April-May (Frederiksen & Rasmussen, 2006). Dispersal happens with explosive seeds, but also with extensive clonal growth via rhizomes (Berg & Redbo-torstensson, 1998). The leaves are made up of long, red petioles, bearing three heart-shaped leaflets folded to the midrib, and widely known for their popular sour taste (Šircelj *et al.*, 2010). It is known as one of the most shade-tolerant plant species (Jankowska-Blaszczuk & Daws, 2007) and can be found mostly in moist woodlands (Šircelj *et al.*, 2010).

Viola reichenbachiana

Viola reichenbachiana (L., Violaceae), also known as Early-dog Violet, is a cleistogamous forb, reaching heights of 5-20 cm and flowering in May (Frederiksen & Rasmussen, 2006). The leaves are heart-shaped and hairy (Yousefi *et al.*, 2012). Dispersal happens firstly with explosive seeds, and subsequently through myrmecochory (Prinzing *et al.*, 2008). *Viola reichenbachiana* grows in woodlands, and has a massive distribution range from central Europe to the Himalayas (Prinzing *et al.*, 2008; Yousefi *et al.*, 2012). Furthermore, *V. reichenbachiana* and *V. riviniana* often share the same habitat with hybridisation occurring in the contact zones allowing gene flow between closely related species (Migdałek *et al*., 2017).

Figure 6. *Viola reichenbachiana.*

GENETICALLY HERITABLE PHENOTYPIC VARIATION IN UNDERSTOREY HERBS

One focus of my dissertation is genetically heritable phenotypic variation, as it is an important level of biodiversity and a pre-requisite for adaptation of species to their environment through natural selection. Although relationships between intraspecific trait variation and environmental variables, as well as phenotypic plasticity, have been studied in forest understorey herbs before, studies testing for the genetic basis of phenotypic variation are rare. Moreover, relationships of genetically based phenotypic trait variation with management-related variables have so far not been investigated.

Noteworthy previous studies include a series of publications on *A. nemorosa* and *M. effusum* (De Frenne *et al.*, 2011b; De Frenne *et al.*, 2012). In a reciprocal transplant along a latitudinal gradient, these two study species showed genetically based phenotypic variation in various life history traits. Moreover, evidence of local adaptation was observed in the measured fitness traits. These studies are the first examples to show that forest understorey herbs show genetically based phenotypic variation among populations and that they can be locally adapted to their environment along latitudinal gradients. However, the question remains whether understorey herbs display phenotypic variation and local adaptation to forest management.

Another complementary study by Willems et al. (2021), studied the flowering phenology of forest understorey herbs and how it was affected by forest management and forest structural attributes. Intra-specific trait variation in flowering time caused understorey herbs to flower two weeks later on plots with high management intensity compared to plots with low management intensity. Effects of forest structural attributes and microclimate on flowering time was also found (Willems *et al.*, 2021). However, since these observations are based on field observations alone, we cannot infer whether these responses are purely plastic or whether they have a genetic basis.

Intra-individual variation

The study of the variation occurring within the repeated homologous structures in plants (e.g., leaves) (Pérez-Harguindeguy et al., 2013) is known as intra-individual variation or sub-individual variation (Herrera, 2009), and is a relatively new field of study within ecology. Studies show that intra-individual variation can have effects on the individual's fitness and underlying functional mechanisms (Herrera *et al.*, 2015; Herrera, 2017). Additionally, most of the variation in homologous structures, e.g., fruits, leaves, and seeds, have been found at the intra-individual hierarchical level of biological organisation.

Clonal plant species generally produce several ramets, that have the potential to be propagated into independent individuals and that can vary phenotypically. The ability to study intra-individual variation in clonal plant species at several hierarchical levels of biological organisation makes them therefore the ideal study organisms. However, measurements of evolutionary responses are considerably complex in clonal plants, as they can experience natural selection in traits on all hierarchical levels (Fischer & van Kleunen, 2001). Intra-individual variation can be subject to natural selection within the different levels (Fischer & van Kleunen, 2001), but the genetic basis of intra-individual variation as well as the genetic variation in the strength of phenotypic plasticity of intra-individual variation remained an open question. Furthermore, in the face of climatic changes, it is also worth investigating how intra-individual variation may facilitate individuals and populations to adapt to environmental changes plastically and genetically.

STUDY SYSTEMS

Various methods exist to study genetically heritable phenotypic variation. The study species used throughout the experiments are all able to produce vegetatively to different extents. This allows for unique opportunities, as clonal propagation can be applied to obtain genetically identical ramets as study replicates.

Common gardens

A common garden is an experimental setup, in which researchers transplant individuals originating from different locations together in one place under identical conditions (Figure 7). By doing this, observed morphological and phenological differences among individuals with different origins, can be ascribed to having a genetic basis (Turesson, 1922). Common gardens not only allow us to investigate the genetic variation of ecologically important traits, but also the genetic variation in the strength of phenotypic plasticity to experimental treatments (West-Eberhard, 2003). Common gardens also provide the opportunity to apply various treatments to answer more specific research questions. If the overall requirement of identical environments, this time within the treatments, are upheld, common gardens could be used e.g., to simulate future climatic conditions by applying treatments such as increased temperatures or drought, *et cetera*.

Figure 7. Illustration of common garden experiment in Frankfurt a. M.

Reciprocal transplants

Reciprocal transplants are the “golden standard” to study local adaptation (Johnson *et al.*, 2022). In a reciprocal transplant, individuals originating from different locations are transplanted back to their local conditions and to all other locations as well. These transplantations allow for the separation of environmental and source of origin effects, and the interactions between them. When conducting reciprocal transplants, local adaptation can be viewed in several ways. Comparisons between individuals transplanted ”Home vs. Away” compare the fitness of a population at its home site to the fitness when transplanted into other habitats, and any observed variance across populations are often due to habitat effects (Blanquart *et al.*, 2013). In ”Local vs. Foreign” comparisons, the fitness of a focal population at home is compared to the fitness of other populations transplanted into the focal patch, and any observed variance across populations is more likely to be due to deme quality effects (Blanquart *et al.*, 2013). Furthermore, a “Sympatric vs. Allopatric” comparison investigates the general performance of individuals growing in their local environment with individuals growing outside their local environment, while taking both habitat and deme quality into account (Adiba et al., 2010). Local adaptation is often visualized through reaction norms, however reciprocal transplants conducted along continuous environmental gradients, can accommodate for investigation into the strength of local adaptation, an approach not often seen in reciprocal transplant studies.

SCOPE AND OUTLINE

Biodiversity Exploratories

My project took place within the Biodiversity Exploratories (BE), a large-scale platform for ecological research in Germany. The BE consists of three regions, along a latitudinal gradient in Germany: The Schwäbische Alb in the south, the Hainich-Dün in the middle, and the Schorfheide-Chorin reserve in the north (Figure 8). Within each region are 50 forest plots, harbouring strong differences in forestry and management intensities as well as great variation in their landscape structure and climatic conditions. All 150 forest plots were established between 2006 – 2009, and continuous collection of site-specific data has been ongoing ever since. All data for all plots have been made available through the publicly available database BExIS2.

Schwäbische Alb (ALB) is a UNESCO biosphere reserve in the south of Germany and is characterized by calcareous bedrock. ALB has the highest elevation of the three regions, up to 860 m, and despite being the most southern location, it has the coldest annual mean temperature of 6-7 °C and a mean annual precipitation of 700-1000 mm (Fischer *et al.*, 2010). However, despite the large annual precipitation, water is quite limited due to soil conditions. The mountain ranges are rich in limestone, and in combination with steep reliefs, erosion leads to shallow soils with low water storage potential. ALB is mostly characterized by small-scale mosaic forests (Fischer *et al*., 2010).

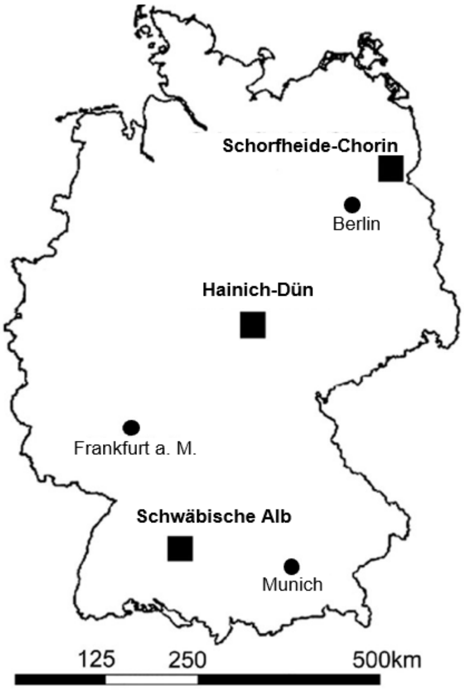

Figure 8. Overview of the three regions of the Biodiversity Exploratories in Germany (indicated by squares), and nearby major cities (indicated by circles). Adapted from Fischer *et al*., 2010.

Hainich-Dün (HAI) is located in the middle of Germany and is also characterized by calcareous bedrock. HAI has a maximum elevation of 550 m, a mean annual temperature of 6.5-8 °C, and a mean annual precipitation of 500-800 mm. HAI is one of the largest connected deciduous forest areas in Germany, mainly characterized by extensive beech forests, and is dominated by selection forest management (Fischer *et al*., 2010).

Schorfheide-Chorin (SCH) is a UNESCO biosphere reserve, located in the northern part of Germany, and is a young glacial landscape with a maximum elevation of 140 m, it has the warmest mean annual temperature of the three regions with 8-8.5

°C, and it is located in one of the driest regions in Germany with a mean annual precipitation of 500-600 mm (Fischer *et al*., 2010). SCH is characterized by sandy areas, hilly grounds, and numerous lakes throughout the landscape. The majority of the forests are characterized as age-class, consisting of old natural beech forests, mixed forests but also intensively managed pine monocultures (Fischer *et al*., 2010).

Aims

I conducted a series of experiments to answer several research hypotheses and to fill knowledge gaps in current plant evolutionary ecology, in particularly with regards to forest understorey herbs within the context of forest management. I sampled all individuals from the forest plots of the BE regions. All forest plots are 100 × 100 m and inside the plots is a fenced area consisting of a weather station (with varying intra-plot location).

Chapter 1

First, I conducted a common garden experiment using all five study species to assess genetically based phenotypic variation, a pre-requisite study since forest understorey herbs have not yet been studied within the context of adaptation to forest management. I wanted to answer the following questions as part of **Chapter 1**:

1.1) Do populations of forest understorey herbs differ genetically in phenotypic variation. If so, how does among-population phenotypic trait differentiation correlate with forest structural attributes and microenvironmental variables?

1.2) Does the forest structure have a direct effect on genetically based phenotypic variation in forest understorey herbs, or indirect effects through the microenvironment?

I sampled genets of each species, originating from 70-100 populations and measured both morphological and phenological traits in the growing period of 2022. To specifically investigate the direct and indirect effects of the forest attributes and microenvironmental variables on the measured plant traits, I performed structural equation modelling (SEM) separately for each trait and species.

Chapter 2

Second, I conducted a reciprocal transplant experiment along a silvicultural management intensity gradient, using the species *A. nemorosa* and *M. effusum* to study local adaptation to forest management, and to answer the following questions as part of **Chapter 2**:

2.1) Are populations of forest understorey herbs locally adapted to forest management and the local environmental variables?

2.2) Does the strength of local adaptation vary between species with contrasting dispersal rates and gene flow?

2.3) Are unoccupied forest sites unsuitable for colonization, or are they unoccupied due to limited dispersal abilities of the species?

I sampled genets from 17-27 plots, distributed along a management intensity gradient. A novel feature of this experiment was that each genet was propagated into ramets, which would be used for the transplants. Working with ramets allows for taking the genetic basis into account when reciprocally transplanting. This means that, whatever trait mean variation was observed among replicates was not due to genetic differentiation among the individuals but could be ascribed to plastic response. Furthermore, a specific performance score was obtained by first ranking and then summing up the phenological stage of every ramet within a pot. The rankings were as follows: 0 = no plant visible, 1 = emerging ramets, 2 = vegetative ramet, 3 = ramet with flowering bud, 4 = ramet currently flowering or had been flowering (Figure 9).

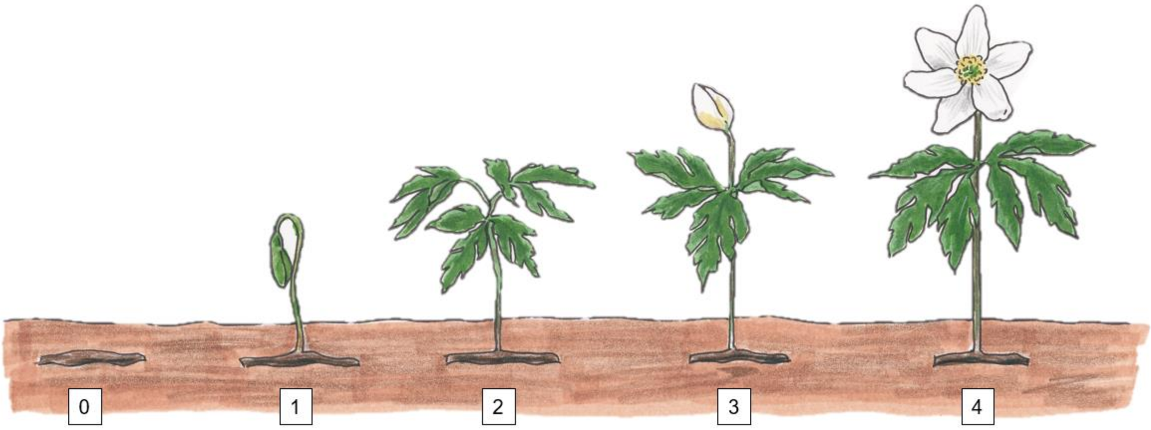

Figure 9. Performance ranking of *Anemone nemorosa* as an example. A ranking of 0 was given if the individual was dead or had no above-ground biomass, 1 for emerging ramets, 2 for a vegetative leaf individual, 3 for an individual with a flowering bud, and 4 for an individual currently flowering or had been flowering.

To test for local adaptation to forest management (SMI) and the local environmental variables, we applied models for the measured traits and the environmental difference (Δ) between the origin sites and transplant sites. Both linear and quadratic terms of the environmental variables were included in the model. The hypothesis behind including both terms is to test for the potential of both local adaptation (Figure 10, solid line) but also phenotypic plasticity (Figure 10, dashed line). Individuals transplanted back to the origin (sympatric) will be located at 0 on the x-axis, as they experience no differences in the environment. If individuals are locally adapted, they would be expected to have a higher trait performance when transplanted sympatrically, and a lower trait performance when transplanted allopatrically.

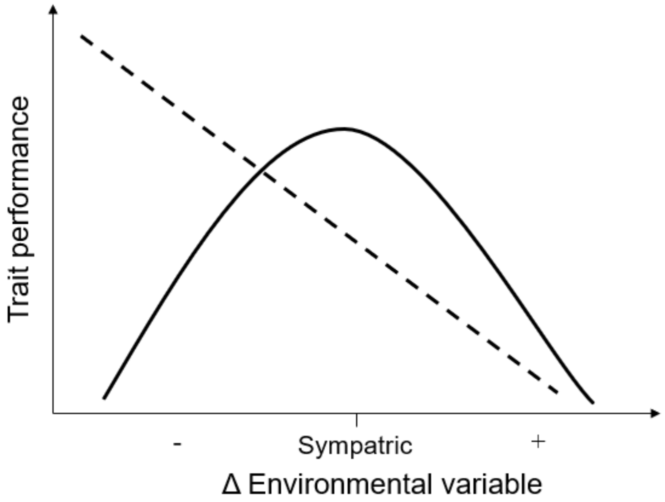

Figure 10. Schematic figure representing possible outcomes of local adaptation and phenotypic plasticity. Solid concave line represents local adaptation, linear dashed line represents phenotypic plasticity.

Chapter 3

And third, my last experiment was another common garden experiment, this time with treatments applied to simulate future climatic conditions for exploring intra-individual variation. I used *G. odoratum* as a study species to answer the following questions as part of **Chapter 3**:

3.1) Which hierarchical level of biological organisation best explains measured intra-individual variation in vegetative traits? Does this variation have a genetic basis?

3.2) Does forest management and the related microenvironmental factors at the population of origin explain (in part) intra-individual trait variation?

3.3) How does experimentally induced drought and earlier shading drive plastic responses in intra-individual trait variation?

I sampled genets from 21 plots, distributed along a management intensity gradient. Again, I propagated each genet into four ramets. Each ramet would experience one of the four treatments: control, drought, early shading or a combined treatment, in a full factorial experimental design. The early shading treatment was applied approximately two weeks before natural leaf out of the surrounding trees in the area to mimic the observed phenological mismatch (Heberling *et al*., 2019). As a measurement of intra-individual variation in **Chapter 3**, we calculated the coefficient of variation (CV) as the standard deviation divided by the mean of a specific trait. Intra-individual variation in leaf length and leaf width could be calculated at each hierarchical level of organisation (intra-population, intra-genet, intra-ramet and intra-shoot). However, intra-individual variation of plant height could not be calculated at the intra-shoot level, as there only exists one measurement of plant height per shoot, and no standard deviation can be calculated.

GENERAL DISCUSSION

By conducting three experiments with novel experimental designs, a complementary discussion of the results can be made. I will first give a quick overview of the final experimental sizes prior to addressing each specific aim individually, followed by discussing the results in the context of adaptive evolution, forest management and microclimatic change. After, I address the methodological limits of all results and I will finish with an outlook and an overall conclusion.

MAIN FINDINGS

The experiments for **Chapter 1** and **Chapter 2** lasted from November 2020 to August 2022 for a duration of two years, whereas the experiment for **Chapter 3** ran from November 2020 to August 2021 for a duration of one year. During the experiments, some individuals suffered from mortality, making the exclusion of some individuals necessary. The final numbers of populations, genets and ramets included in the experiments out of the total number sampled can be viewed in Table 1.

Table 1. Final number of populations, genets and ramets for each study species included in the manuscripts and publication.” – “ denotes that the species was not included in the study. Black font indicates the final number included in the studies, whereas the grey font represents the initial sampled amount.

POPULATION LEVEL	**Chapter 1**	**Chapter 2**	**Chapter 3**
Anemone nemorosa	68 (100)	27 (27)	-
Galium odoratum	69 (90)	-	21 (27)
Milium effusum	67 (79)	17 (18)	-
Oxalis acetosella	72 (75)	-	-
Viola reichenbachiana	70 (70)	-	-
TOTAL	128 (135)	39 (39)	21 (27)

GENET LEVEL			
Anemone nemorosa	126 (600)	151 (162)	-
Galium odoratum	166 (540)	-	71 (108)
Milium effusum	178 (474)	78 (108)	-
Oxalis acetosella	361 (450)	-	-
Viola reichenbachiana	298 (420)	-	-
TOTAL	1 129 (2 484)	229 (270)	71 (108)

RAMET LEVEL			
Anemone nemorosa	-	420 (648)	-
Galium odoratum	-	-	517 (540)
Milium effusum	-	166 (432)	-
Oxalis acetosella	-	-	-
Viola reichenbachiana	-	-	-
TOTAL	-	586 (1 080)	517 (540)

Some species were more prone to suffer mortality, sometimes strongly reducing the final number of individuals used in the experiments. Most notably, a warm spell in January 2021 triggered the emergence of *A. nemorosa*, however a deep late frost event in February 2021 caused a big mortality event in the already emerged *A. nemorosa* individuals. Luckily, general mortality was low and evenly distributed among individuals and populations for all species, and only a few populations in **Chapter 3** had to be excluded due to insufficient number of individuals to be able to calculate CV.

Forest management drives evolution of understorey herbs

1.1*) Do populations of forest understorey herbs differ genetically in phenotypic variation. If so, how does among-population phenotypic trait differentiation correlate with forest structural attributes and microenvironmental variables?*

I found that populations of forest understory herbs varied genetically in the measured traits. As the measurements were taken on individuals in a common garden rather under natural conditions, this phenotypic variation reflects genetic differentiation among populations. Populations of understorey herbs differed significantly in emergence (*A. nemorosa* measurement only) and proportion of flowering ramets. Furthermore, I also showed significant interactions between populations and species for flowering start and plant height.

Correlations with forest structural attributes and microenvironmental variables give us a bigger insight as to whether or not the genetic differentiation is a result of adaptation to local conditions. Emergence was significantly affected by soil pH, indicating the importance of the below-ground microenvironment for this specific phenological cue. Similarly, flowering start was also affected by soil pH, in addition to the interaction with species, and the two-way interaction between spring temperature and species. Proportion of flowering ramets only differed significant between species. Finally, plant height showed a significant two-way interaction between soil pH and species, and a marginally significant two-way interaction for percentage of conifer and species. In sum, it is clear from these results that there are immense species differences, and each trait and the effects of the forest structural attributes and microenvironment should be investigated separately for each species to gain a fuller picture.

1.2) *Does the forest structure have a direct effect on genetically based phenotypic variation in forest understorey herbs, or indirect effects through the microenvironment?*

Not all significant relationships and interactions found in the mixed-effects model could also be found in the species-combined structural equation models (SEMs). Due to contrasting species responses to the microenvironment of their origin, the total effect would be cancelled out if analysed together. Therefore, it was necessary to do species-specific SEMs to properly investigate the direct and indirect effects of forest management and microenvironment, respectively.

First, emergence, a trait only measured for *A. nemorosa*, occurred later in individuals originating from more acidic soils, but earlier flowering start of *O. acetosella* was generally found in individuals from more acidic soils. Furthermore, I found individuals of both *O. acetosella* and *G. odoratum* to reduce their amount of flowering ramets and invest more in vegetative production, when originating from acidic soils. This suggests that soil pH is an important driver of trait variation (Gentili *et al.*, 2018), possibly due to its effect on nutrient availability. Moreover, individuals of *O. acetosella* originating from warmer forest plots, flowered later in the common garden. Ultimately, individuals originating from colder plots, often coniferous, might have developed a phenological cue for flowering start at lower temperatures, which persists when the individuals are transplanted to the common garden in Frankfurt, where the phenological cue might be triggered earlier in the year due to warmer temperatures. Additionally, *A. nemorosa* individuals originating from colder plots also invested more into vegetative ramets and reproduction, potentially a strategy to conserve resources for warmer spring temperatures, when pollinator activities are higher (Hegland *et al.*, 2009). Plant height in *G. odoratum* was the only measured trait directly affected by a forest structural attribute, structural complexity.

Despite showing evidence for genetic differentiation, *M. effusum* and *V. reichenbachiana* did not show any influence of the microenvironmental variables from their sites of origin. This could be due to various reasons, one being that the environmental variables that could explain the genetic differentiation were not investigated, or that the observed variation is neutral with respect to any environmental variables. Furthermore, as soil pH from the plot of origin often had great impact on the measured traits for several species, the importance of soil pH as a key soil property was highlighted. Based on my results, I conclude that the effects of forest management on plants growing under natural conditions in the field (Willems *et al.*, 2021) are at least partly a result of genetic differentiation and thus forest management can have evolutionary consequences for forest understorey herbs on small spatial scales.

Forest understorey herbs are locally adapted to forest management intensity

2.1*) Are populations of forest understorey herbs locally adapted to forest management and the local environmental variables?*

Through a reciprocal transplant experiment, I showed that *A. nemorosa* was locally adapted to structural complexity in all measured traits. Structural complexity is a representation of vertical and horizontal heterogeneity, which is often lacking in forests with high management intensity. A heterogeneous environment can provide selection for phenotypic variation within plant traits and ultimately help conserve genetic diversity. Additionally, *A. nemorosa* also showed local adaptation of plant height to SMI. However, most relationships were linear, indicating that trait performance was not highest at sympatric sites but rather that phenotypic plasticity acts along environmental gradients. Individuals transplanted to colder, more acidic plots with lower management intensity would increase in trait performance. Acidic soils can be advantageous for plant growth, as micronutrients are more easily available to plants compared to neutral-alkaline soils (Lončarić *et al*., 2008). The advantages of acidic soils might “spill over” into the colder plots, as these are tightly connected with the percentage of conifers on the forest plot. Similarly, individuals transplanted to lower management intensity (SMI) plots had an increased performance in all traits. The lack of a heterogeneous microenvironment in intensively managed forests restricts phenotypic plasticity, which becomes evident by the poorer performance of *A. nemorosa* individuals transplanted to high management intensity plots. Another potential explanation for the many linear relationships could be the co-gradient variation hypothesis (Conover & Schultz, 1995), stating that favourable environmental conditions can cause increased trait expression along an environmental gradient. Plastic responses are necessary for rapid responses to a changing environment, but too much plasticity can also shield populations from selection, hindering genetic adaptation to local conditions. *Milium effusum* mostly showed significant linear relationships of the measured traits to soil pH, indicating phenotypic plasticity acting along this gradient. No local adaptation was detected in *M. effusum* otherwise.

In sum, my results suggest that local adaptation and phenotypic plasticity help plants to adjust along environmental gradients. Forest understorey herbs show local adaptation to structural complexity after two growing seasons, but linear relationships to forest management and other forest structural attributes, suggest that they should be viewed independently from structural complexity, and that understorey herbs potentially rely more on plasticity for adaptation along a management gradient.

2.2) *Does the strength of local adaptation vary between species with contrasting dispersal rates and gene flow?*

“Sympatric vs Allopatric” categorical comparisons for each trait yielded no differences, potentially due to the forced artificial categories of near and far allopatric sites on an initial continuous gradient of SMI. However, when taking the results from the environmental differences into account (discussed in **2.1**), I show that it is actually only *A. nemorosa* that has significant concave relationships, implying that during the two measured growth periods, it was only *A. nemorosa* that managed to show local adaptation. This could suggest that stronger local adaption might be associated to life strategies prioritising slow clonal spread, as they cannot rely on quick dispersal, cf. the wind-dispersed seeds of *M. effusum*. In fact, *M. effusum* showed maladaptation in two traits. A significant convex relationship was observed between number of ramets and environmental differences in structural complexity, suggesting that individuals performed worse when transplanted sympatrically back to their home site. Another significant convex relationship for *M. effusum* was also observed in “Sympatric versus

Absent” transplants between plant height and soil pH. The observed maladaptation provides an interesting aspect to my experiment. However, the explanation for these observations of maladaptation is unclear and could potentially be caused by distorted trait means based on individuals after a mortality and selection event, further discussed in **2.3**.

2.3) *Are unoccupied forest sites unsuitable for colonization, or are they unoccupied due to limited dispersal abilities of the species?*

In the “Sympatric vs Absent” comparisons, I observed strong local adaption in *A. nemorosa*, where the individuals always performed significantly better at sympatric sites. In contrast, *M. effusum* performed better at foreign sites. There could be several explanations for this. First of all, it is important to keep in mind, that *M. effusum* went through a mortality event in both years, especially at foreign sites. The mortality could potentially have functioned as a strong selection event. If only the strongest individuals survived and established, these individuals would on average perform better than individuals transplanted sympatrically where all genotypes, even the weaker ones, would have a higher chance at surviving. However, *M. effusum* individuals transplanted to foreign sites might also have increased performance due to a release from local enemies, such as soil pathogens and below-ground herbivores (De Frenne *et al.*, 2014). Results showing *M. effusum* performing better at foreign sites where the species did not naturally occur suggest that after successfully going through the establishment phase, individuals can on average perform well. Despite *A. nemorosa* performing better at sympatric sites, individuals still survived at some specific foreign sites. This indicates that unoccupied forest sites are indeed suitable habitats for future potential establishment of both species, and species might be limited by dispersal rather than establishment.

Overall, this might indicate that establishment limitation is dictated by dispersal abilities and gene flow, which in turn affects the ability to locally adapt. *Anemone nemorosa* relies heavily on clonal spread and has a very low dispersal ability, and the evolutionary strategy of *A. nemorosa* would be to adapt to sites where they occur, rather than to try and migrate away if the environment becomes unsuitable. On the other hand, *M. effusum* has a high dispersal rate, which gives it a greater ability to migrate and establish in new environments. With higher dispersal rate and gene flow, *M. effusum* would benefit from greater amounts of phenotypic plasticity, allowing for greater performance at foreign sites.

The role of intra-individual variation

3.1) *Which hierarchical level of biological organisation best explains measured intra-individual variation in vegetative traits? Does this variation have a genetic basis?*

My results showed that out of all the hierarchical levels of biological organisation that was included in the study, intra-shoot, the lowest level, represented most of the total variation found in the measured traits leaf length and leaf width. This is in line with previous studies investigating intra-individual variation in vegetative traits (Herrera *et al.*, 2015; Herrera, 2017). Since intra-shoot level is not possible to calculate for plant height, the next lowest level, intra-ramet, represented most of the total variation in this trait. As an alternative explanation for genetically based differences, the intra-individual variation could also be a result of epigenetically or perhaps transgenerational plasticity-based phenotypic variation (Herrera *et al.*, 2022; Sobral & Sampedro, 2022). Additionally, general patterns of the highest hierarchical levels, intra-region and intra-population, having the best Akaike information criterion (AIC) goodness of fit, suggest that it is on these levels that genetic basis and differentiation of intra-individual variation operate.

3.2) *Does forest management and the related microenvironmental factors at the population of origin explain (in part) intra-individual variation?*

Out of various microenvironmental factors, I found intra-individual variation in plant height of *G. odoratum* to mainly correlate with soil temperature from the plot of origin, indicating that intra-individual variation can evolve and may be adaptive. My results showed that individuals originating from colder plots of origin, would increase their variation of height, which could potentially be an evolved way to optimize light capture under light-limited conditions. Limited light availability is often associated with cold, coniferous plots (Willems *et al.*, 2021), supporting the notion of a stress-induced variability hypothesis stating that abiotic stress increases variation (Kuppler *et al.*, 2020). Furthermore, the significant effect of soil temperature on intra-individual variation in plant height indicates that intra-individual variation may ultimately play a key role in adaptation to long-term microclimatic conditions (May *et al.*, 2017).

3.3) *How does experimentally induced drought and earlier shading drive plastic responses in intra-individual trait variation?*

Intra-individual variation responded plastically to drought and early shading treatments depending on the trait and hierarchical level under investigation, suggesting functional changes to improve light capture and reduce evapotranspiration. Variation in leaf length increased when exposed to 2-weeks-earlier shading, indicating a rapid phenotypic change in response to environmental drivers. During a combination of both drought and early shading treatment, increased intra-individual variation was observed, potentially a result of plant responses in contrasting directions (Winn, 1996; Sun *et al.*, 2021), but ultimately benefitting the performance of the individuals by facilitating drought tolerance.

For intra-individual variation to be considered an independent measurable trait, able to evolve unconstrained with respect to trait means, the relationship between trait means and intra-individual variation needed to be investigated (Herrera, 2017). In my study, several significant relationships between these two components were found, both linear and quadratic, suggesting that they are interdependent but act in complex relationships, and that one could drive the other. The quadratic relationship suggests a stabilizing selective regime (March-Salas *et al.*, 2021), where intermediate intra-individual variation would be the optimal strategy for plants to invest in. Ultimately, as intra-individual variation may be partially genetically based, this also means that they can be under the influences of neutral or selective evolutionary forces.

ADAPTIVE EVOLUTION IN FOREST UNDERSTOREY HERBS

Adaptive evolution produced by natural selection can be recognized in the form of genetic differentiation, local adaptation, and even phenotypic plasticity. Throughout this dissertation, I addressed adaptive evolution in forest understorey herbs from various angles and approaches through a series of experiments, which were often tightly linked to forest management and the microenvironment.

Interesting notions to think about are the trajectory of future adaptive evolution, and what we can expect based on my conducted experiments presented here. If anthropogenic activities and land use changes continues to increase, we can expect increasing forest management activities leading to a higher percentage of conifers, homogeneous forest interiors and microenvironments, but also more unpredictable and extreme climatic events. The observed species responses throughout the three presented experiments suggest that phenotypic plasticity will be a key component for species to respond fast and appropriately, ultimately leading to evolution of trait means to changing climatic conditions. To be more specific, higher intra-individual variation and stronger phenotypic plasticity in all traits improve the species' ability for rapid responses to a changing environment.

In a study by De Frenne et al. (2011), local adaptation of *A. nemorosa* and *M. effusum* was observed along a latitudinal gradient. Furthermore, individuals of *A. nemorosa* benefitted from simulated higher temperatures, indicating that climate warming might increase future competitive ability and colonization rates (De Frenne *et al.*, 2011b). However, it is important to note that the existence of past local adaptation does not necessarily mean that rapid adaptation to future climatic change can also occur. Perennial species with a longer life span often have a slower rate of adaptive evolution than the predicted rate of climate change (Jump & Peñuelas J, 2005) and might therefore rely more on their adaptive plasticity, which can itself also be subject to evolution (Matesanz *et al.*, 2010). A long-term study by Anderson et al. (2012) showed that the strength of directional selection and the predicted evolutionary responses have increased the last 30 years in response to climatic changes. This indicates that perennial plants might be able to evolve rapidly in response to selection imposed by anthropogenic climate change if strong selection and moderate heritability is present (Anderson *et al.*, 2012). More recently, Van Daele et al. (2022) conducted genomic analyses on the forest herb *Primula elatior*, showing a lack of adaptations to climate extremes. Forest understorey herbs occurring in the buffered climatic conditions of the forest interior, might experience a reduced selection pressure imposed by the climate (van Daele *et al.*, 2022). Contrasting results and predictions of the adaptive evolution in forest understorey herbs underline the importance of continuing research elucidating this topic further, especially in the face of ongoing climatic changes and increasing land-use.

In **Chapter 1**, I show that soil pH from the plots of origin affects the traits measured in the common garden and thereby the genetic basis. However, the directional effect varies among species. In **Chapter 2**, I detected strong phenotypic plasticity in both species along a soil pH gradient, where individuals always performed better in acidic soils. In **Chapter 3**, I show that intra-individual variation is affected by the origin of the individuals as soil temperature showed significant negative correlations with intra-individual variation. As soil temperature is likely affected by forest management, forest management of the plot of origin can therefore be said to indirectly affect intra-individual variation measured in the common garden, indicating that intra-individual variation can evolve and may be adaptive. Below-ground microclimatic conditions specifically, have been shown through this dissertation to be very important and have impacts on the adaptive evolution of forest understorey herbs.

Chapter 2 shows clear impacts of forest management in the form of local adaptation. Forest understorey herbs showed local adaptation to structural complexity, a forest structural attribute representing heterogeneity, and high structural complexity is often associated with less intensively managed forests. Furthermore, *A. nemorosa* also showed local adaptation in plant height to forest management intensity itself, underlying the big impact that forest management has on understorey herbs. However, I reveal phenotypic plasticity to be important and the primary solution and coping mechanism when individuals were transplanted along environmental gradients.

In **Chapter 3**, I show that intra-individual variation responds plastically to drought and early shading treatments. As I applied these treatments to simulate future climatic conditions, it proves that intra-individual variation has an important role in genetic adaptation and in plastic adjustments to a rapidly changing environment and future climatic conditions. As a result of the shading treatment, intra-individual variation increased indicating a rapid phenotypic change in response to environmental drivers (**Chapter 3**), but as temperatures increase it is likely that the phenological mismatch will grow bigger and understorey herbs will need increased amounts of phenotypic plasticity to be able to respond appropriately.

In sum, through a series of experiments presented in my dissertation, I show that forest understorey herbs are not only locally adapted to forest structural attributes as dictated by management regimes, but even the genetic basis of understorey herbs is affected by forest management. This calls for substantial consideration when planning future management actions.

FOREST MANAGEMENT IMPLICATIONS

Future forest management decisions can have great direct impact on several microenvironmental variables which in turn affect understorey herbs. One example is the buffering effect caused by the “forest roof”, which can be compromised by direct management effects to the canopy cover, resulting in forest interior temperatures to increase (De Frenne *et al.*, 2021). However, future forest management actions could also help alleviate some of the climate change effects by focusing on creating a more heterogenous mosaic of forest management within regions. With the results from **Chapter 1**, it was possible to establish genetic differentiation of forest understorey herb populations at small spatial scales, and therefore the genetic variation can be conserved at small spatial scales. As all the observed genetic variation occurs within the BE regions, forest management actions can be altered specifically to each regional scale, with the aim to create heterogeneity, to allow selection for different plant traits and thus to conserve genetic diversity and evolutionary potential of the understorey herb populations. However, I recommend caution when applying regular disturbances as a results of forest management activities, as this could accelerate tree die-off and widespread crown defoliation, increase risk of forest fires, and induce physiological stress, making trees more susceptible to pests (Figure 11).

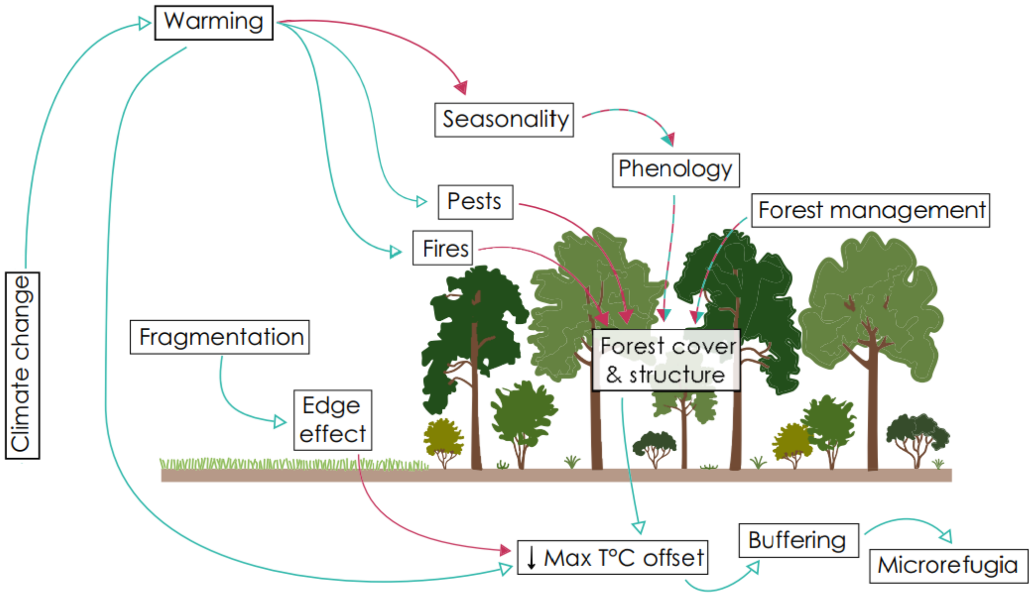

Figure 11. Macroclimate change effects on temperate forest microclimate. Blue arrows indicate a positive effect, red arrows a negative effect, and mixed arrows a mixed effect. Figure adapted from De Frenne *et al.*, 2021.

Temperate forests can provide microrefugia during climate change and buffer against increasing temperatures (De Frenne *et al.*, 2021), and their importance in the global climate system and climate change mitigation is therefore intensively discussed (Thom *et al.*, 2018). However, it is not only the macroclimate that will be affected by future climatic conditions, but also the microclimate, as climate warming affects microclimates and microrefugia by influencing the forest composition and structure indirectly (Figure 11 (De Frenne *et al.*, 2021)). In a response to microclimate warming in forest interiors, thermophilization could occur, shifting the community towards more warm-adapted species. In contrast, cold-adapted species would have to adapt to future climatic conditions, as temperate forests buffering climatic changes would potentially only function as a temporary “hold-out” and thus possibly create an extinction debt (Makishima *et al.*, 2021).

Ultimately, from the point of view of increasing the evolutionary potential of forest understorey herbs, I suggest that future forest management regimes need to be diversified in order to create heterogeneous forests at smaller regional scales, preserving intraspecific genetic diversity. Diverse forests representing various forest management practices likely promotes genetic diversity in forest understorey herbs, providing the necessary genetic material for future evolutionary adaptation.

METHODOLOGICAL LIMITS & OUTLOOK

Methodological limits of the already conducted experiments are often tightly associated with the improvements and future perspectives that would be addressed in an outlook, and I will therefore address them combined for each experiment.

First of all, I initially included *Brachypodium sylvaticum* as a study species. However, due to failed establishment in the pots after the initial transplant, I unfortunately had to exclude this species completely from the experiment.

Methodological improvement of **Chapter 1** could have come in the form of more overlapping trait measurements. The only measurement identical for all the study species was the flowering period in the form of flowering start and flowering end. The number of ramets was obtained by various means, as *O. acetosella* does not produce countable ramets, and for *V. reichenbachiana* I used stolons as a proxy for ramets instead. Furthermore, plant height as a measurement did not make ecological sense for all species and I only measured plant height for *O. acetosella* to calculate a frustrum volume, but never for *V. reichenbachiana*, as both species rely more on horizontal spread than vertical growth. In an effort to create more consistency and robustness of future studies, overlapping measurements, ecologically relevant for all study species should be carefully considered.

Not all species were affected by the investigated forest structural attributes or microenvironmental variables. This does not necessarily mean that they are not affected at all directly by forest management or indirectly by the microenvironment, but maybe that I simply did not include the variables that affect species like *M. effusum* and *V. reichenbachiana*. Future research should consider other forest structural attributes or microenvironmental variables that could potentially affect the genetic basis of forest understorey herbs. This could better the understanding of how forest management can affect the evolution of understorey herbs.

Lastly, a Q_{ST}-F_{ST} analysis (Q_{ST}= quantitative genetics, F_{ST} = fixation index for a single locus) to infer past selection on phenotypic traits could yield valuable hints on adaptation. A Q_{ST}-F_{ST} analysis would allow us to compare neutral genetic differentiation sequenced from e.g., leaf material, with measurements of genetically based phenotypic trait differentiation from a common garden experiment.

An establishment phase of the individuals is often necessary for reciprocal transplant studies before any meaningful results are visible. Indeed, in **Chapter 2**, I observed no significant differences in the first year of measurements for either species. This is likely because the individuals were still establishing in their transplanted environment, and time was still too short to accumulate phenotypic differences that reflect adaptation after transplantation. Long-term reciprocal transplant experiments (Bennington *et al.*, 2012; Johnson *et al.*, 2022) have shown that sometimes local adaptation is only evident after several years of measurements. Indeed, in the second year of measurements, significant differences were present, especially for ‘Sympatric vs Absent’ comparisons.

The inclusion of more study species covering the whole dispersal rate and gene flow gradient in **Chapter 2** could also have been a great advantage and would have allowed for further investigation regarding dispersal *versus* establishment limitations. As for now, even though the two study species *A. nemorosa* and *M. effusum* have contrasting dispersal rates and gene flow, the comparisons between two species only are not enough to establish it as an explanation for the observed species differences.

Figure 12. Illustration of taking measurements for Chapter 3.

High mortalities occurred for individuals in **Chapter 3**, often within individuals from a subset of plots. Mortality due to extreme events is often unforeseeable or can often happen inexplicably, or due to the study species being sensitive to transplantation from the wild to pots in a common garden setting. However, as the coefficient of variation is used as a measurement for intra-individual variation (Figure 12), some genets and even populations unfortunately had to be excluded from the datasets as it was not possible to calculate the CV based on a single value.

Lastly, a commentary was published about the published **Chapter 3**, highlighting next steps that are necessary to advance the field of intra-individual variation (Sobral, 2023). Expanding the study to include trans-generational measurements on intra-individual variation would provide valuable information on the role of epigenetic variation in response to environmental change across generations. Furthermore, more traits, especially related to sexual reproduction, might be worth including (Figure 12). Intra-individual variation is known to be greater in vegetative traits compared to, e.g., flower traits (Herrera, 2017). Additionally, comparisons between species relying on sexual production to various degrees can provide insight on how intra-individual variation is varying across generations depending on the reproductive system.

CONCLUSIONS

As a first step, I show that forest understorey herbs genetically differentiate in ecologically important traits among the sampled populations (**Chapter 1**). This genetic differentiation and the evolution thereof seem to be strongly determined both directly by forest structural attributes and indirectly through their influence on the microenvironment. In order to maintain intraspecific genetic diversity of understorey herbs, it is therefore crucial that forest management practices themselves remain spatially diverse.

Understorey herbs, more specifically *A. nemorosa*, showed local adaptation to forest structural attributes and forest management (**Chapter 2**). However, the strength of adaptation and the type of adaptation vary among species, the experienced management intensity and the associated microenvironment. If available, phenotypic plasticity seems to be a first responder to a changing environment, acting along environmental gradients as experienced in the forest.

Furthermore, I prove that Intra-individual variation at the lowest hierarchical levels of organisation host the majority of the variation within traits and should therefore not be ignored as an important component for adaptation and appropriate trait responses to environmental change and future climatic conditions (**Chapter 3**). I show that phenotypic plasticity is used as a flexible strategy to adapt to varying environmental conditions. By adjusting leaf characteristics and the variation within, plants can optimize their fitness in response to changes in light availability, water availability, and overall seasonal cues.

Concluding, understorey herbs have evolved various adaptations in response to management practices in temperate forests. The experimental studies on forest understorey herbs included in my dissertation, have contributed to the understanding of their ecological traits and functioning thereof, of adaptive strategies, and of responses to environmental changes. This improved knowledge is crucial for effective forest management strategies promoting conservation of biodiversity and genetic diversity in understorey herbs, and for predicting the impacts of ongoing climatic changes on these important plant communities.

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ANNEX

CHAPTER 1

Original article:

Forest management drives evolution of understorey herbs

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Abstract

1. Forest management has a strong impact on the forest structure and subsequently on the biotic and abiotic forest understorey environment. Forest understorey herbs can thus be expected to evolutionary respond to management-induced environmental variation (provided sufficient time for adaptation), but this has been little tested to date.
2. Here we use a common garden, to test for genetically based variation in phenotypic traits in populations of forest herbs sampled along a forest management intensity gradient. Five different herbaceous species were sampled from 70-100 populations in three regions in Germany and were tested for genetically based variation in flowering start, proportion of flowering ramets, and plant height. Additionally, we investigated the effects of management-induced environmental variation and performed structural equation modelling to study how forest management drives trait differentiation *via* its effects on the microenvironment.
3. We found that the studied forest understorey herbs varied genetically in the measured functional and phenological traits among the sampled populations. Forest management likely affected the traits in various directions and strengths depending on the species, either directly through variation in forest structural attributes or indirectly through changes in the microclimatic environment on the forest floor.
4. *Synthesis:* We show that forest management can have evolutionary consequences for forest understorey plants. In an applied context, diverse forest management actions within landscapes thus creates heterogeneity that selects for different plant traits and thus helps conserving genetic diversity.

Keywords: common garden, evolutionary ecology, forest structure, genetic differentiation, intraspecific trait variation, microclimate, structural equation modelling.

Introduction

In our rapidly changing world, land use has been recognized as having the strongest impact on species and genetic diversity (IPCC, 2014a; Tobias *et al.*, 2021). The present state of temperate forests in Europe is a product of decisions made by former and current management, rather than natural factors and progression (Spiecker, 2003). Current silvicultural management practices have a profound impact on forest ecosystems, as they can affect forest structure, for instance by altering crown projection area, structural complexity, and tree species composition. Large, dense tree crowns cast shade, buffer against warming and reduce daytime summer temperatures inside forests (De Frenne *et al.*, 2013). Furthermore, light availability is highly dependent on the composition of tree species, which in turn depends on site-specific climatic factors such as water availability (Aussenac, 2000). Existing silvicultural management and conservation actions supporting coppicing, selective tree extractions or other actions can lead to canopy openings, potentially causing nutrient depletion of the soil, a decreasing biomass pool, increasing nitrogen release in the soil, but also warming of the forest floor, likely causing changes in the composition of understorey herb species (De Frenne *et al.*, 2013).

Forest understorey herbs play a key role in forest ecosystems (Augusto *et al.*, 2003; Gilliam, 2007; Landuyt *et al.*, 2019), harbouring the highest species diversity among all strata in temperate forests (Gilliam, 2007). In temperate forests, the phenology of early-growing understorey herbs should be attuned to abiotic cues, such as spring temperature, in order to prevent early frost or to finish their reproductive cycle before tree leaf out (Heberling *et al.*, 2019). High management intensity for example can result in delayed flowering in understorey herbs (Willems *et al.*, 2021). More specifically, low spring temperatures were associated with intensely managed conifer plantations, with understorey herbs flowering on average two weeks later than in unmanaged forests. However, this study is based on field observations only, and thus cannot infer whether the results are due to phenotypic plasticity or genetic differentiation.

Forest management, its influence on the forest floor environment, as well as how it impacts adaptation of understorey herbs, are still underexplored. Soil pH is highly influential on plant phenology and growth, and affects traits such as germination (Gentili *et al.*, 2018), plant height and number of flowers (Jiang et al., 2017). Some aspects of acidic soils can be advantageous for plant growth, as more micronutrients are available to plants compared to neutral-alkaline soils (Loncaric et al., 2008). Studies have found that plants germinate and flower earlier on acidic soils, but species differences in the number of flowers (Gentili *et al.*, 2018; Sercu *et al.*, 2021). In general, functional trait responses to forest structural variation and microenvironment depend on the phenology and distribution of the forest understorey herb, and can therefore be highly species-specific (De Frenne *et al.*, 2009; Blondeel *et al.*, 2020; Willems *et al.*, 2021).

One response of forest understorey plants to silvicultural management actions and microenvironmental changes, is through the employment of genetically based phenotypic variation to respond appropriately to stress and disturbances (Lemke *et al.*, 2015). Mutations, genetic drift and gene flow are neutral processes affecting genetically based phenotypic variation and population genetic structure, and genetic variation within populations is a necessary prerequisite for adaption to and persistence under changing conditions (Lemke *et al.*, 2015). As sessile organisms, plants are vulnerable to strong environmental changes, and especially forest understorey herbs with limited dispersal capabilities may likely be adapted to very specific and local environments. Because forest offer relatively temporally stable environments, we could expect understorey herbs to adapt evolutionary to spatial environmental variation rather than to evolve phenotypic plasticity (Alpert & Simms, 2002; Valladares *et al.*, 2007). However, few studies have looked at genetic adaptation of forest understorey herbs to their local environment (Møller *et al.*, 2023b). A useful strategy to investigate evolutionary adaptation is by applying a common garden experiment, because it can reveal genetic differentiation among sampled populations, followed by correlative approaches to test for associations between trait variation and environmental variation at sites of population origins (Turesson, 1922a; Scheepens & Stöcklin, 2013).

Here we established a common garden using five understorey herbs sampled across a forest management intensity gradient, to (1) investigate whether populations of forest understorey herbs differ genetically in phenotypic variation; (2) If so, how does among-population phenotypic trait differentiation correlate with forest structural variables and microenvironmental variables? And (3) does the forest structure have a direct effect on genetically based phenotypic variation in forest understorey herbs, or indirect effects through the microenvironment?

Material & methods

Study species

For this study, we chose five perennial forest understorey herbs, four forbs – *Anemone nemorosa, Galium odoratum, Oxalis acetosella* and *Viola reichenbachiana* – and one grass – *Milium effusum* (Fig. 1).

Anemone nemorosa (Ranunculaceae) and *O. acetosella* (Oxalidaceae) are both early-flowering, small forbs, reaching heights of 5-25 cm. They rely heavily on clonal spread (Frederiksen & Rasmussen, 2006). By contrast, *G. odoratum* (Rubiaceae) and *V. reichenbachiana* (Violaceae) flower later, grow taller, reach heights of 10-30 cm, and rely more on seed dispersal (Frederiksen & Rasmussen, 2006). Lastly, *M. effusum* (Poaceae) is a tall-growing grass, reaching heights of 50-150 cm, and relies heavily on wind-dispersed seeds (De Frenne *et al.*, 2011b). All species can spread vegetatively to some degree and vary in colonization rates (Brunet *et al.*, 2012).

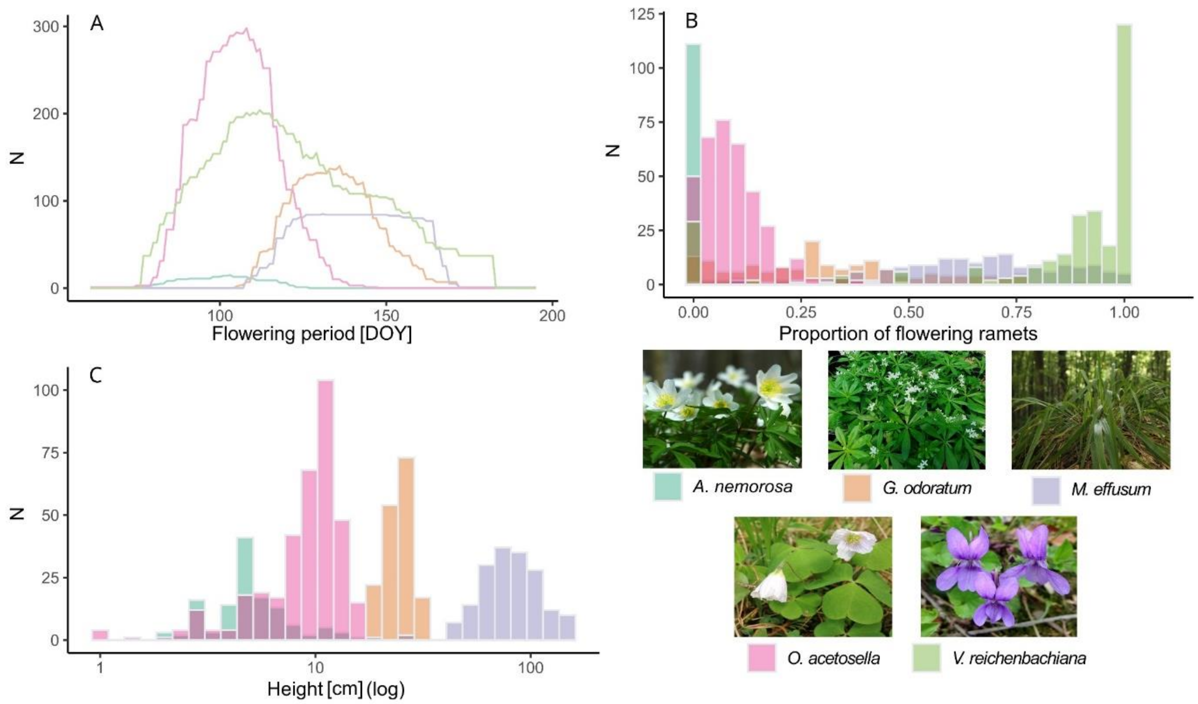

***Figure 1.** Trait distribution and number of observations (N) for (A) flowering period [DOY], (B) proportion of flowering ramets, and (C) plant height [cm] (x-axis logged) for Anemone nemorosa, Galium odoratum, Milium effusum, Oxalis acetosella, and Viola reichenbachiana. Photo credits: Franziska Willems, Pieter De Frenne & Charlotte Møller.*

Experimental set-up

In 2020, plants were sampled from plots set up within the framework of the Biodiversity Exploratories (www.biodiversity-exploratories.de), a large-scale platform for ecological research (Fischer *et al.*, 2010). The Biodiversity Exploratories contains three regions across Germany: Schwäbische Alb, Hainich-Dün, and Schorfheide-Chorin (Fischer *et al.*, 2010). Each region consists of 50 forest plots, *i.e.,* 150 plots across the three regions.

In May 2020, all forest plots were visited. Six genets were sampled for each species (hereafter referred to as individuals), whenever they were occurring on a plot. To avoid sampling genetically identical plants, each individual was sampled with a minimum distance of 10 m to the next sampled plant. Individuals were planted in multitrays (51.5 cm width, 33.5 cm length, 5.5 cm deep, 54 pots per tray) filled with potting soil (“CL T torffrei”, Einheitserde, Sinntal-Altengronau, Germany) for root establishment and growth. In November 2020 all individuals were transferred into 1.5 L pots with potting soil (“Typ T, Struktur 1B”, Hawita, Vechta, Germany). In spring 2021, all pots were relocated to a shading house (Götsch & Fälschle, Alerheim, Germany), under common-garden conditions, situated at the Goethe University Frankfurt (Frankfurt am Main, Germany). All individuals received water *ad libitum* during the whole experiment.

Plant measurements

In total, we sampled 2474 individuals: 606 *Anemone nemorosa,* 545 *Galium odoratum,* 467 *Milium effusum,* 430 *Oxalis acetosella,* and 426 *Viola reichenbachiana*, from 70-100 populations. However, mortality caused heavy species-dependent losses after transplantation, mainly in February 2021 due to a period of deep frost following a warm spell. One year later, in the growing period of 2022, we tracked flowering phenology, counted ramets and measured vegetative traits on the remaining 1,129 individuals: 126 *A. nemorosa*, 166 *G. odoratum*, 178 *M. effusum*, 361 *O. acetosella* and 298 *V. reichenbachiana* (See Table S1 for distribution between populations of sampled individuals).

Phenological measurements were taken three times per week (every Monday, Wednesday, and Friday). Since *Anemone nemorosa* is a vernal geophyte, emergence date was recorded only for this study species, while flowering start and flowering end were recorded for all study species. Dates were transformed into Julian days (day of the year; DOY). Flowering peak was calculated using one-hot encoding (Lantz, 2019), transforming the data to be categorical in order to count how many individuals, from each species, were flowering on a given day. To calculate the proportion of flowering ramets, the number of flowering ramets and total number of ramets were counted for *A. nemorosa*, *G. odoratum* and *M. effusum*. To infer the total number of ramets for *O. acetosella*, we applied the formula for the frustrum of cone volume measurement:

$$^{1}/_{3}\,\pi * h(R^2 + Rr + r^2) \tag{1}$$

with *h* being the height of the individual, *R* being the radius of the lower base, and *r* being the radius of the upper base. We consider that each leaf connects to a rhizome and is therefore potentially independent; all leaves were counted for a subset of 12 pots, based on a subjective stratified sampling of small, intermediate, and large plants, and a regression between the frustum volume (Eq. (1)) and the number of leaves ($R^2 = 0.93$, see Fig. S1) was used to predict the number of leaves in the remaining pots. The proportion of flowering ramets was then calculated as the total number of flowers divided by the predicted number of leaves. For *V. reichenbachiana*, major stems were used as a proxy for ramets, and the number of major stems and number of major flowering stems were counted and used to calculate the proportion of flowering stems, hereafter referred to as proportion of flowering ramets. Plant height was measured for *A. nemorosa*, *G. odoratum*, *M. effusum* and *O. acetosella*, but not for *V. reichenbachiana* as height is not a meaningful variable in this species.

Forest structural attributes and microclimatic variables for each population of origin were extracted from the BExIS2 (www.bexis.uni-jena.de) database, maintained by the Biodiversity Exploratories (Fischer *et al.*, 2010), for the years 2018 and 2019, *i.e.,* previous to the plants being sampled, to catch relatively recent environmental variation leading up to the sampling in 2020. Structural complexity index (SCI), an index quantifying stand structural complexity based on the fractal dimension of cross-sectional polygons (Ehbrecht *et al.*, 2017), crown projection area (m^2/ha) of mature trees, and the percentage of conifers based on crown projection, was obtained for all sampled plots. These forest attributes were selected because they characterize stand structure and are expected to have an influence on the biotic and abiotic microclimatic conditions, as well as on light availability (Willems *et al.*, 2021). In addition, we also obtained soil pH and the annual mean spring temperature 10 cm above the forest floor (calculated as the average of March, April, and May of 2018 and 2019) as microenvironmental variables.

Data analyses

All statistical analyses were conducted with R version 4.1.2 (R Core Team, 2021b). First, to test for population differences in genetically based phenotypic variation, we ran linear mixed-effect models (LMM) for all traits, using the function “lmer” from the package “lme4” (Bates *et al.*, 2007). The measured plant traits were used as the response variables, population, species, and their interaction as the explanatory variable, and region was included as a random factor.

Second, to investigate the effect of forest attributes and microclimate from the population of origin on the measured traits in the common garden, we ran LMMs with structural complexity, crown projection area, percentage of conifers, soil pH, spring temperature, and the two-way interaction between species and all the aforementioned as explanatory variables. Region and population were used as random nested structure (Region/Population). LMMs were run using the function “lmer” from the packages “lme4” (Bates *et al.*, 2007). The model test results were obtained by applying the “Anova” function from the package “car” (Fox *et al.*, 2012). Post hoc tests were applied to investigate significant differences among species using the package “lsmeans” (Lenth, 2016).

Third, to investigate the direct and indirect effects of the forest attributes and microclimatic variables on the measured plant traits, we performed structural equation modelling (SEM) separately for each trait. Due to the significant species interactions in the LMMs of the second study question, SEMs were done separately for each species. We used the package “piecewiseSEM” (Lefcheck, 2016), which is particularly suitable for handling LMMs with random factors. In each individual SEM, a measured plant trait (flowering start, proportion of flowering ramets or plant height) was included as the response variable, with region and population used as random nested structure. Spring temperature and pH were included as explanatory variables for the plant trait, but also as response variables for structural complexity, crown projection area and percentage of conifers. Additionally, these forest structural attributes were included as direct explanatory variables for the measured plant traits and could also correlate amongst each other. The complete data set varied between SEMs, but each overall path model was evaluated based on a Fischer’s C statistic and on its degrees of freedom and was accepted as an adequate model if the baseline model had a P-value > 0.05. Marginal and conditional R^2-values were calculated for each response variable, the former describing the proportion of variance explained by fixed factors alone, the latter describing the variance explained by both fixed and random factors. Despite starting with a model based on *a priori* knowledge of direct and indirect interactions, pathways with a P-value > 0.1 were removed from the final visualization of the SEMs.

Results

Population differentiation and effect of forest structure and microclimatic variables on plant traits

Emergence of *A. nemorosa* showed a marginally significant difference among populations of origin (Table 1). Flowering start and plant height differed significantly among populations of origin across species as well as in the interaction between populations and species (Table 1). The proportion of flowering ramets varied significantly among populations across species (Table 1).

Flowering start was, across all species, significantly delayed in plants from sites with higher soil pH. Additionally, the significant interaction between soil pH and species indicated that species differed in their relationship between flowering start and soil pH (Table 2). A post-hoc Tukey test revealed that *V. reichenbachiana* significantly differed from both *O. acetosella* and *M. effusum* (Tukey P-value = <0.001 and 0.018, respectively) and this species – in contrast to the cross-species pattern – showed delayed flowering start in plants from sites with lower soil pH (Fig. S2). Furthermore, the interaction between mean yearly spring temperature and species, showed significant variation among flowering start in species (Table 2), whereas *M. effusum* was found to significantly differ from *O. acetosella* (Tukey P-value = 0.040), and stood out from the other species by delaying flowering start in individuals originating from colder sites (Fig. S3).

The proportion of flowering ramets showed significant variation among species. A post-hoc Tukey test revealed that *A. nemorosa* significantly differed from *V. reichenbachiana* and *G. odoratum* (Tukey P-values = <0.001 and 0.040, respectively). Similarly, *O. acetosella* significantly differed from *V. reichenbachiana, G. odoratum,* and *M. effusum* (Tukey P-values = <0.001, 0.026, and <0.001, respectively), and together with *A. nemorosa*, showed the lowest proportion of flowering ramets (Fig. 1, Fig. S4). At the other end of the scale, *M. effusum* showed a higher proportion of flowering ramets, significantly differing from the rest of the species (Tukey P-value: *G. odoratum* = <0.001), except for *V. reichenbachiana*. *Viola reichenbachiana* had the

Table 1: *Results of mixed-effects models of emergence (for Anemone nemorosa only), flowering start, plant height and the proportion of flowering ramets in five different forest understorey herbs explained by population, species, and their interaction as fixed factors, and region as random variable. Chi-square (*X^2*) test values, degrees of freedom (df), and P-values (P) are given. Significant P-values are indicated in bold followed by * (<0.05), ** (<0.01) and *** (<0.001), and marginally significant values are indicated in bold followed by ·.*

	Emergence			**Flowering start**			**Proportion of flowering ramets**			**Plant height**		
	X^2	df	P	X^2	df	P	X^2	df	P	X^2	df	P
Population	85.40	67	**0.064 ·**	160.0	116	**0.004 ****	162.5	127	**0.018 ***	258.3	123	**<0.001 *****
Species				0.946	4	0.918	3.839	4	0.428	3.135	3	0.371
Population * Species				220.7	164	**0.002 ****	216.5	0.439	0.439	214.6	148	**<0.001 *****

Table 2. *Results of mixed-effects models of emergence (for Anemone nemorosa only), flowering start, plant height and the proportion of flowering ramets in five different forest understorey herbs explained by species, environmental variables, and their interactions with species as fixed factors, and with region and populations included as nested random variables. For continuous variables, upwards arrows (↑) indicate positive estimates and downwards arrows (↓) indicate negative estimates. Chi-square (X^2) test values, degrees of freedom (df), and P-values (P) are given. Significant P-values are indicated in bold followed by * (<0.05), ** (<0.01) and *** (<0.001), and marginally significant values are indicated in bold followed by* **·**.

		Emergence				**Flowering start**				**Proportion of flowering ramets**				**Plant height**		
		X^2	df	P		X^2	df	P		X^2	df	P		X^2	df	P
Species							4	0.997		212.78	4	**<0.001 *****		1.792	3	0.617
pH	↓	5.663	1	**0.017 ***	↓		1	**0.021 ***	↓	2.032	1	0.154	↓	1.633	1	0.201
Spring temperature	↓	1.210	1	0.271	↓		1	0.428	↓	0.416	1	0.519	↓	1.254	1	0.263
Conifer	↓	0.113	1	0.737	↓		1	0.952	↓	2.237	1	0.135	↓	1.373	1	0.241
Structural complexity	↓	0.646	1	0.422	↓		1	0.799	↓	0.535	1	0.465	↓	0.977	1	0.323
Crown projection area	↑	0.061	1	0.805	↓		1	0.331	↓	0.653	1	0.419	↓	0.020	1	0.888
pH * Species							4	**<0.001 *****		1.243	4	0.871		13.35	3	**0.004 ****
Spring temperature * Species							4	**0.047 ***		3.440	4	0.487		3.495	3	0.321
Conifer * Species							4	0.770		8.825	4	**0.066 ·**		7.146	3	**0.067 ·**
Structural complexity * Species							4	0.954		0.781	4	0.941		5.667	3	0.129
Crown projection area * Species							4	0.958		1.448	4	0.836		1.377	3	0.711

highest proportion of flowering ramets of all species, and significantly differed from all species except *M. effusum* (remaining Tukey P-value to be explained: *G. odoratum* = <0.001). Lastly, *G. odoratum* differed significantly from all species and took an intermediate position (Fig. 1, Fig. S4).

The significant interaction between soil pH and species for plant height showed that species differed in their relationship between plant height and soil pH (Table 2). Here, *M. effusum* differed significantly from *A. nemorosa* and *O. acetosella* (Tukey P-value = 0.019 and 0.005, respectively), and increased its height with increasing soil acidity (Fig. S5).

Direct and indirect effects of forest management on understorey herbs

Multiple SEMs revealed that understorey herbs were affected directly by forest structural attributes and indirectly through the microclimate. However, these effects showed different patterns among species. We observed in almost all individual SEMs that our forest structural attributes had strong relationships with each other. Low structural complexity was associated with small crown projection area (found in 100% of SEMs; Fig. 2-4) and with a low percentage of conifers (100%) present at the sites of origin, and a large crown projection area was itself also associated with a high percentage of conifers (57.1%). Although significant paths varied depending on the specific trait or species investigated, we saw a general pattern of soil pH decreasing with percentage of conifers (78.6%) but increasing with structural complexity (14.3%) and crown projection area (35.7%). Furthermore, spring temperature decreased with increasing percentage of conifers (78.6%) and structural complexity (21.4%) but increased with increasing crown projection area (78.6%).

Emergence date of *A. nemorosa* was negatively associated with higher soil pH (Fig. 2A), meaning that individuals originating from populations with high soil pH emerged earlier. Individuals of *O. acetosella* flowered later if originating from populations with a high soil pH or high spring temperature (Fig. 2D). The flowering start of *G. odoratum*, *V. reichenbachiana* and *M. effusum* in the common garden was not significantly affected by any environmental variables from the sites of origin (Fig. 2BCE).

The proportion of flowering ramets decreased with increasing mean spring temperatures in *A. nemorosa* (Fig. 3A), decreased with increasing soil pH in

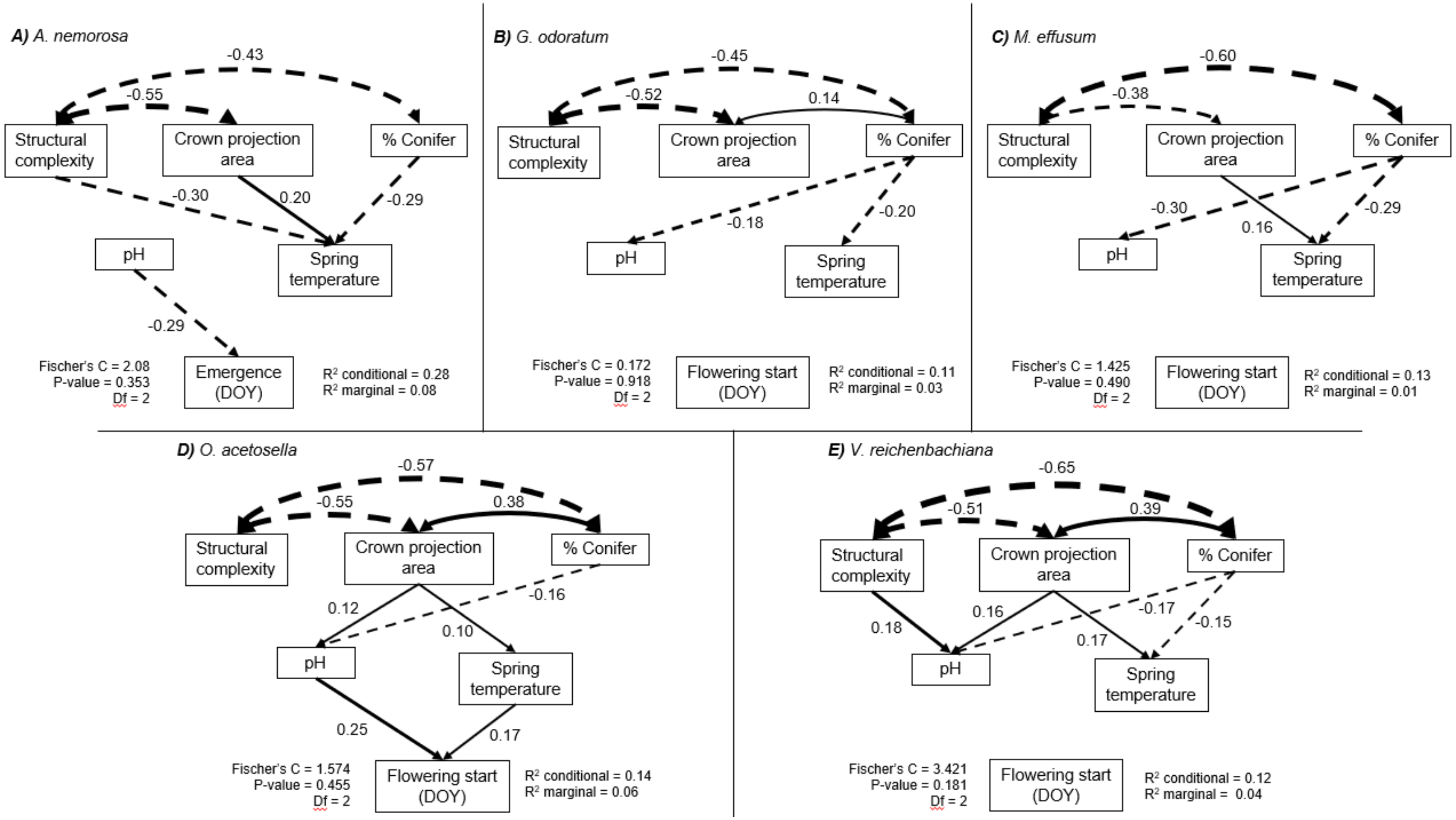

Figure 2. Effects of forest structural attributes and microenvironment of the sites of origin on phenological traits in the common garden. *Shown are the results of the piecewise structural equation model (SEM) for direct and indirect relationships among forest characteristics and microclimatic variables, and the flowering start (DOY) for (A) Anemone nemorosa, (B) Galium odoratum, (C) Milium effusum, (D) Oxalis acetosella, and (E) Viola reichenbachiana. Arrows represent unidirectional and bidirectional relationships among variables; only marginally or more significant paths (P < 0.1) are shown. Solid lines are positive relationships, dashed lines are negative ones. The line thickness is proportional to the magnitude of the standardized estimates, plotted near each line. The* R^2 *values are given for each SEM, along with the overall model fit to the data as Fischer's C, its associated P-value and degrees of freedom (Df).*

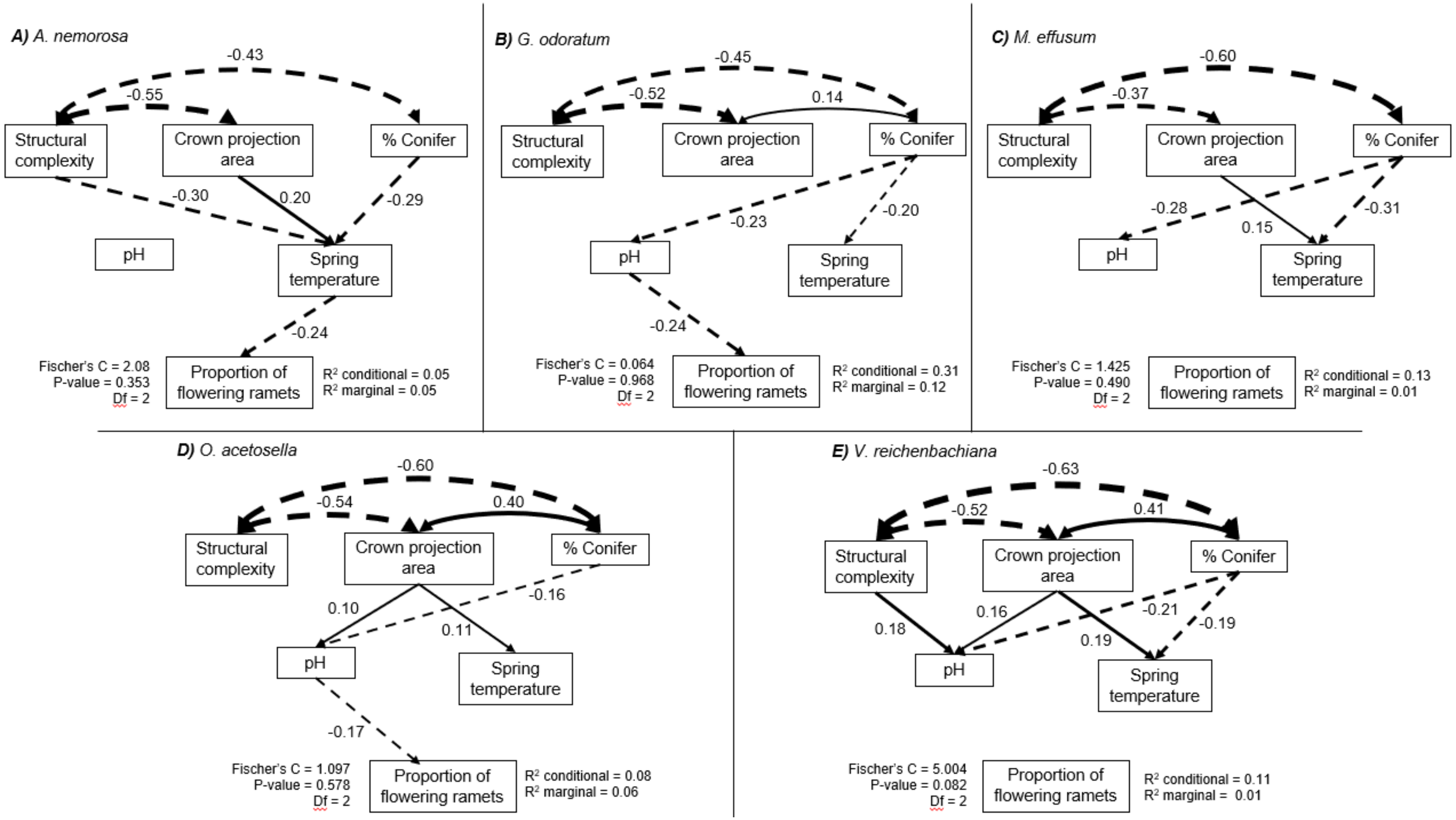

Figure 3. Effects of forest structural attributes and microclimate of the site of origin on flowering proportions in the common garden. *Shown are the results of the piecewise structural equation model (SEM) for direct and indirect relationships among forest characteristics and microclimatic variables, and the proportions of flowering ramets for (A) Anemone nemorosa, (B) Galium odoratum, (C) Milium effusum, (D) Oxalis acetosella, and (E) Viola reichenbachiana. Arrows represent unidirectional and bidirectional relationships among variables; only marginally or more significant paths (P < 0.1) are shown. Solid lines are positive relationships, dashed lines are negative ones. The line thickness is proportional to the magnitude of the standardized estimates, plotted near each line. The* R^2 *values are given for each SEM, along with the overall model fit to the data as Fischer's C, its associated P-value and degrees of freedom (Df).*

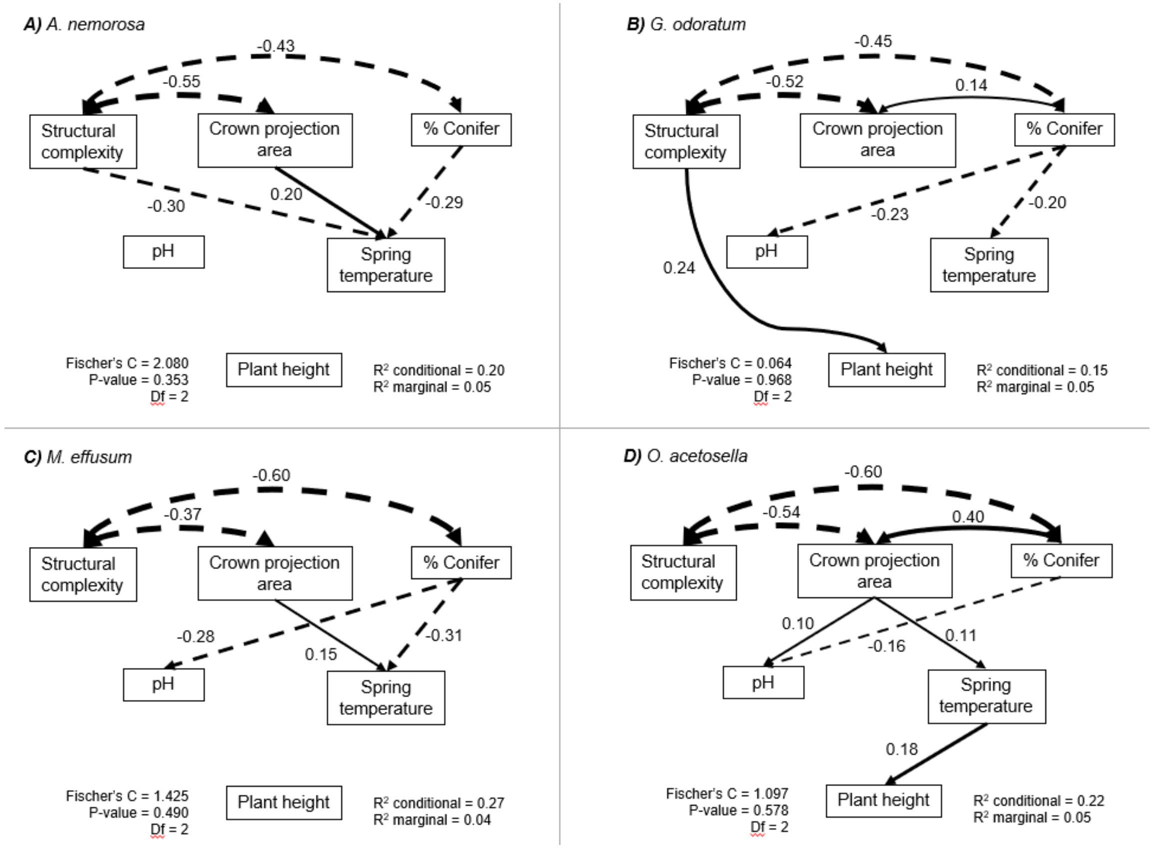

Figure 4. Effects of forest structural attributes and microclimate of the site of origin on plant height in the common garden. *Shown are the results of the piecewise structural equation model (SEM) for direct and indirect relationships among forest characteristics and microclimatic variables, and the proportions of flowering ramets for (A) Anemone nemorosa, (B) Galium odoratum, (C) Milium effusum, and (D) Oxalis acetosella. Arrows represent unidirectional and bidirectional relationships among variables; only marginally or more significant paths ($P < 0.1$) are shown. Solid lines are positive relationships, dashed lines are negative ones. The line thickness is proportional to the magnitude of the standardized estimates, plotted near each line. The R^2 values are given for each SEM, along with the overall model fit to the data as Fischer's C, its associated P-value and degrees of freedom (Df).*

O. acetosella and *G. odoratum* (Fig. 3BD) and was not affected by any variables from the sites of origin for *V. reichenbachiana* and *M. effusum* (Fig. 3CE).

The height of *O. acetosella* individuals increased with increasing mean spring temperatures (Fig. 4D). Additionally, the plant height of *G. odoratum* was directly affected by forest structure, with individuals growing taller if they originated from populations in more structurally complex forests (Fig. 4B). Neither the plant height of *A. nemorosa* nor that of *M. effusum* was significantly affected by any variables from the sites of origin (Fig. 4AC).

Discussion

Our common garden study using five forest understorey herbs revealed genetic differentiation among populations in ecologically important traits. Moreover, direct effects of forest structural attributes as well as indirect effects through forest floor microenvironment influenced variation in these traits, although most responses appeared to be species-specific. Taken together, this suggests that forest management influences the evolution of understorey herbs.

Genetically based phenotypic variation in five forest understorey herbs

We showed that understorey herbs harbour significant variation in functional traits depending on their population of origin. As the measurements were obtained from individuals in a common garden rather than *in situ* in the original populations, this phenotypic variation reflects genetic differentiation among populations (Turesson, 1922a; Scheepens & Stöcklin, 2013), although we should be aware that epigenetic effects may explain part of this variation (Bossdorf et al. 2008). This genetic differentiation could be the result of adaptation to local conditions, so our next steps was to investigate associations between trait values and environmental values, which would support adaptation as the cause for this genetic variation (Scheepens & Stöcklin, 2013). In particular, we investigated associations with forest structural attributes, which are strongly related to forest management decisions, as well as microenvironmental variables that may be influenced by forest management. However, since we did not find overall main effects and since species often differ in the influence of certain environmental factors on plant traits, we will instead focus on the species-specific SEMs revealing contrasting patterns.

The effects of forest structure and microenvironment

Concerning the species-specific SEMs, we will first discuss the general patterns found among the forest structural attributes, before examining the patterns found in the forest floor microenvironment. Lastly, we will dissect each species-specific SEM and discuss how the individual traits are responding to the forest structural attributes and the microenvironment at their sites of origin.

General patterns among forest structural attributes were found across all SEMs. Structural complexity was negatively associated with both crown projection area and the percentage of conifers at the sites of origin. Since coniferous forests are often associated with high forest management intensity (Schall & Ammer, 2013; Willems *et al.*, 2021), we often see cohorts of homogenous coniferous plantations leading to low structural complexity (Pommerening, 2004). Similarly, a large crown projection area, caused by coniferous-dominated forests or a monospecific deciduous forest with large crowns, could be the potential cause of the negative association with structural complexity. In our dataset, conifers had larger crown areas compared to deciduous trees, supporting the positive relationship between percentage of conifers and crown projection area (Grote, 2003). However, it has been shown that the crown radii of mixed stands and conifer stands are generally overestimated, while deciduous crowns are underestimated, due to overlapping of crowns in dense and structurally complex stands (Grote, 2003).

Concurrent with previous studies (*e.g.,* Willems et al. 2021), we found that a higher percentage of conifers decreased mean spring temperatures. Most coniferous trees are evergreen, and hence, the forest floor beneath them is constantly shaded, decreasing the temperature experienced by the understory herb community. Large crown projection areas create a bigger “forest roof”, which not only buffers the temperature on the forest floor and increases mean spring temperatures, but also protects against wind and other mechanistic stresses (De Frenne *et al.*, 2021). Structural complexity creates heterogenous microclimates at fine spatiotemporal scales, and various canopy openings could disrupt the buffering of the “forest roof”, ultimately decreasing spring temperatures (De Frenne *et al.*, 2021).

Conifer species are often planted on acidic soils, but they can also contribute to decrease soil pH even further by promoting soil aluminium contents (Augusto *et al.*, 2003). Stands with a high structural complexity usually harbour deciduous trees or a mix of deciduous and coniferous trees. In other words, they usually have a low percentage of conifers, which could explain the positive relationship with soil pH. Large crown projection area can be characterized by dense and overlapping deciduous tree crowns, and deciduous trees are associated with more alkaline soils compared to coniferous stands (Augusto *et al.*, 2003).

It is crucial for spring geophytes to have appropriate responses to thermal cues to avoid frost damage on the one hand but also utilize the maximum amount of high light on the other hand in order to successfully finish a reproductive cycle before tree leaf out (Heberling *et al.*, 2019). Individuals originating from colder, often coniferous, plots might be adapted to phenological cues at lower temperatures, which persists when they are transplanted to a common garden in Frankfurt, thus having an advance phenological stage in the common garden compared to individuals originating from warmer plots. In line with previous studies (Gentili *et al.*, 2018), *A. nemorosa* individuals originating from more acidic soils emerged earlier in our common garden, establishing the importance of pH as a key factor and soil property. In line with this, the phenological cue for flowering start in *O. acetosella* in our common garden, is later in individuals originating from warmer and more alkaline sites (Sercu *et al.*, 2021), and based on our SEMs these sites mainly consist of deciduous tree species.

The proportion of flowering ramets can be seen as a proxy for the amount of resources an individual chooses to allocate to sexual reproduction *versus* vegetative reproduction. *Anemone nemorosa* favours vegetative reproduction during colder spring temperatures and hence conserves valuable resources for warmer periods when investment in flowers is more successful owing to higher pollinator activity (Hegland *et al.*, 2009). Additionally, we saw that both *O. acetosella* and *G. odoratum* reduce their amount of flowering ramets in individuals originating from acidic soils. Previous studies (e.g., Blondeel et al., 2020; Sercu et al., 2021) report similar results, pointing towards the importance of pH on nutrient availability and the potential importance of pH as a driver of trait variation (Gentili *et al.*, 2018).

Plant height was the only measured trait that was directly affected by forest structural attributes. The structural complexity of the forest canopy heavily influences light availability, a resource causing competition within the understorey herb community, and these biotic forces could potentially cause increased height in *G. odoratum* (Pau *et al.*, 2011). Additionally, increased spring temperatures allow for greater carbon assimilation (Heberling *et al.*, 2019) and for *O. acetosella* to invest in increased plant height.

Why do we not see any influence of forest or microenvironmental variables on the traits of *M. effusum* and *V. reichenbachiana*? Despite showing evidence for genetic differentiation, some species are not responding to the microenvironmental variables from their sites of origin. It could very well be that we picked the wrong environmental variables that could explain it, or that the variation is neutral with respect to any environmental variables.

Previous work by Willems *et al*. (2021) was carried out on the same forest plots, but as the results were based on field observations alone, no conclusion about the genetic basis could be made. With our complimentary work, we now established that the effects of forest management found under natural conditions (Willems *et al.*, 2021) is a result of genetic differentiation and thus evolution at small spatial scales. Since our plants originated from within three regions in Germany, we are not comparing forests from across a large spatial range subject to macroclimatic variation. Instead, all observed genetic variation occurs within these three regions, despite gene flow counteracting population differentiation, especially in the species with higher dispersal abilities (notably *M. effusum*). An important implication is that all observed genetic variation can thus also be conserved at this spatial scale. By applying diverse forest management actions across regional scales, we create heterogeneity that likely selects for different plant traits and thus helps conserving genetic diversity.

Conclusion

In conclusion, forest understorey herbs showed genetic differentiation in ecologically important traits among the sampled populations. Our results suggests that forest management, which strongly determines the forest structural variables included in this study (Ehbrecht *et al.*, 2017), drives the evolution of forest understorey herbs either directly or indirectly through its influence on the microenvironment. Given that forest understorey herbs are important for biodiversity and ecosystem services, it is imperative that future management considers this. Diversifying management practices can affect intraspecific genetic diversity and thus can play a role in the conservation of genetic diversity of understorey herbs.

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Data availability statement

This work is based on data elaborated by the HerbAdapt and Forest Structure (core) projects of the Biodiversity Exploratories program (DFG Priority Program 1374). The datasets are publicly available in the Biodiversity Exploratories Information System (http://doi.org/10.17616/R32P9Q, https://www.bexis.uni-jena.de/ddm/data/Showdata/22766, and https://www.bexis.uni-jena.de/ddm/data/Showdata/31455).

Conflict of interests

The authors have no conflict of interest to declare.

Author contributions

CM, PDF and JFS designed the study. CM conducted the field sampling and the common garden experiment with help from MMS. CM and PDF analysed the data with input from TV, KV and JFS. CM wrote the first draft of the manuscript with all co-authors contributing to revisions. Acquisition of the financial support of the project leading to this publication by PDF and JFS.

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Supplementary Material – Manuscript 1

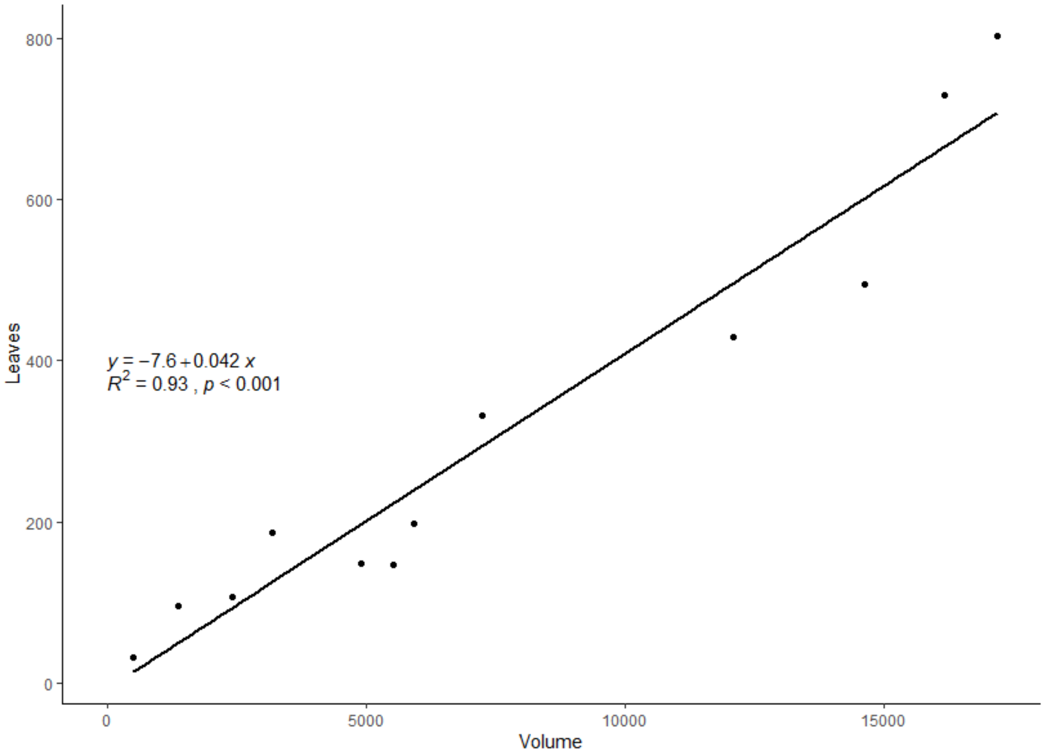

Figure S1. *Linear regression of the number of leaves on plant volume on a subset of 12 Oxalis acetosella individuals. The volume was calculated for each individual in each pot, using the formula for a frustrum volume* $^1/_3\,\pi * h(R^2 + Rr + r^2)$*, and was used in a regression with the number of leaves counted in the same pot as response variable. The resulting regression line equation was then used to predict the number of leaves in the rest of the pots, based on their measured volume.*

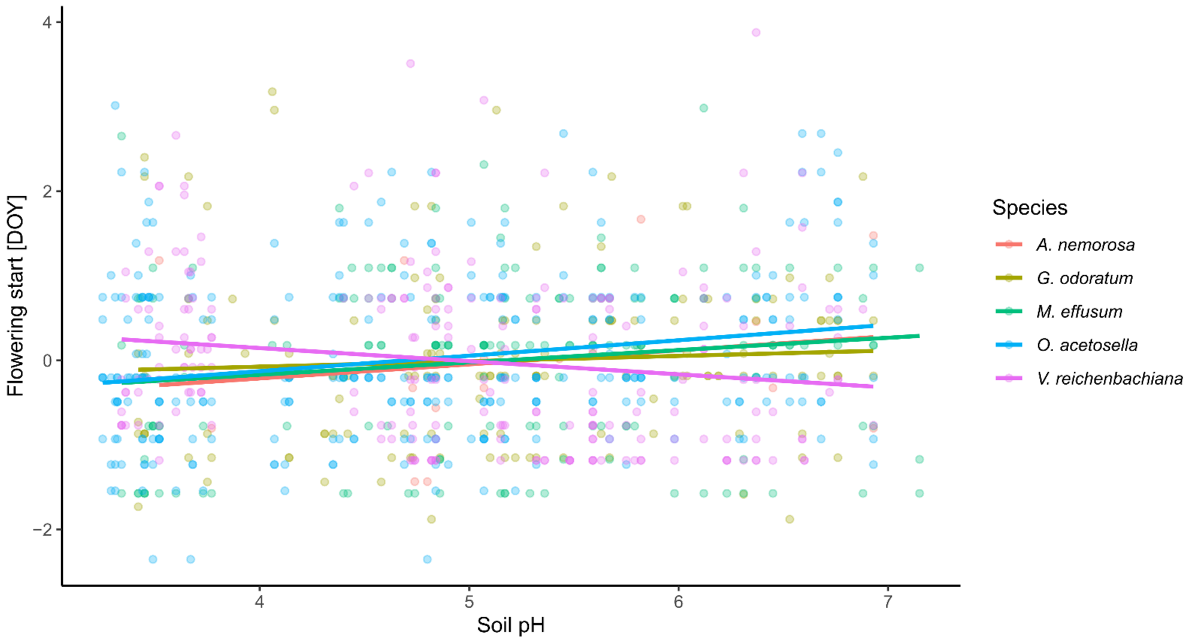

Figure S2. *Linear regressions between flowering start and soil pH from the sites of origin for five species of understorey herbs: Anemone nemorosa, Galium odoratum, Milium effusum, Oxalis acetosella and Viola reichenbachiana.*

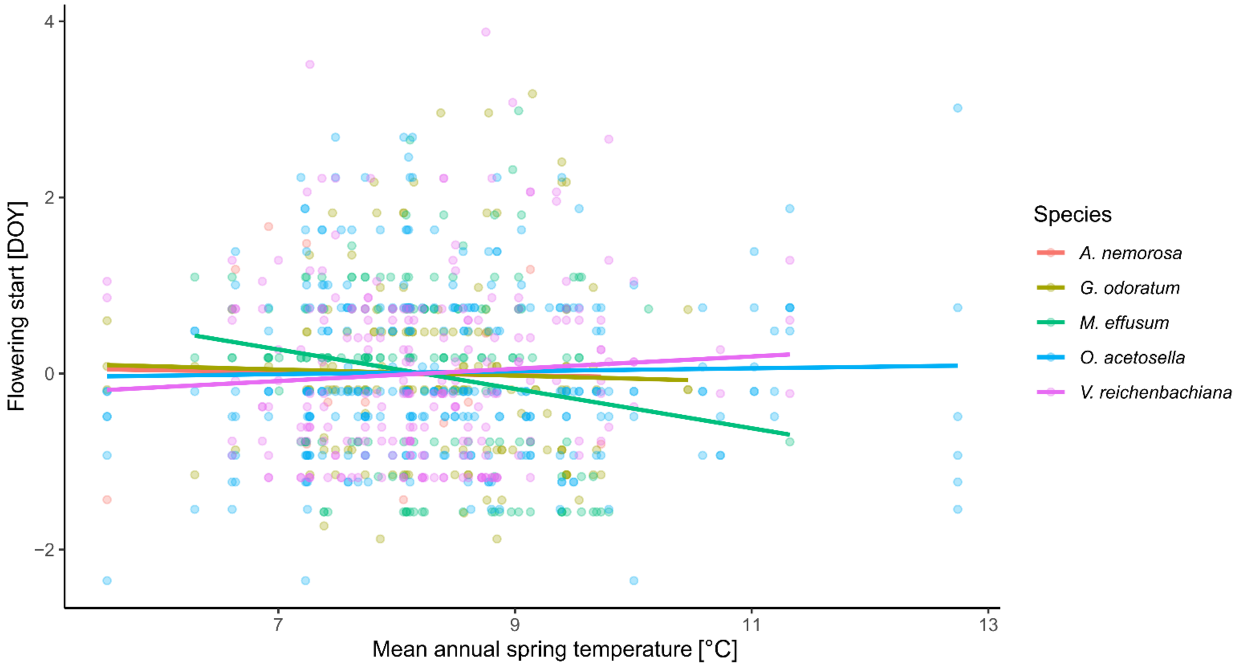

Figure S3. *Linear regressions between flowering start and mean annual spring temperature from the sites of origin for five species of understorey herbs: Anemone nemorosa, Galium odoratum, Milium effusum, Oxalis acetosella and Viola reichenbachiana.*

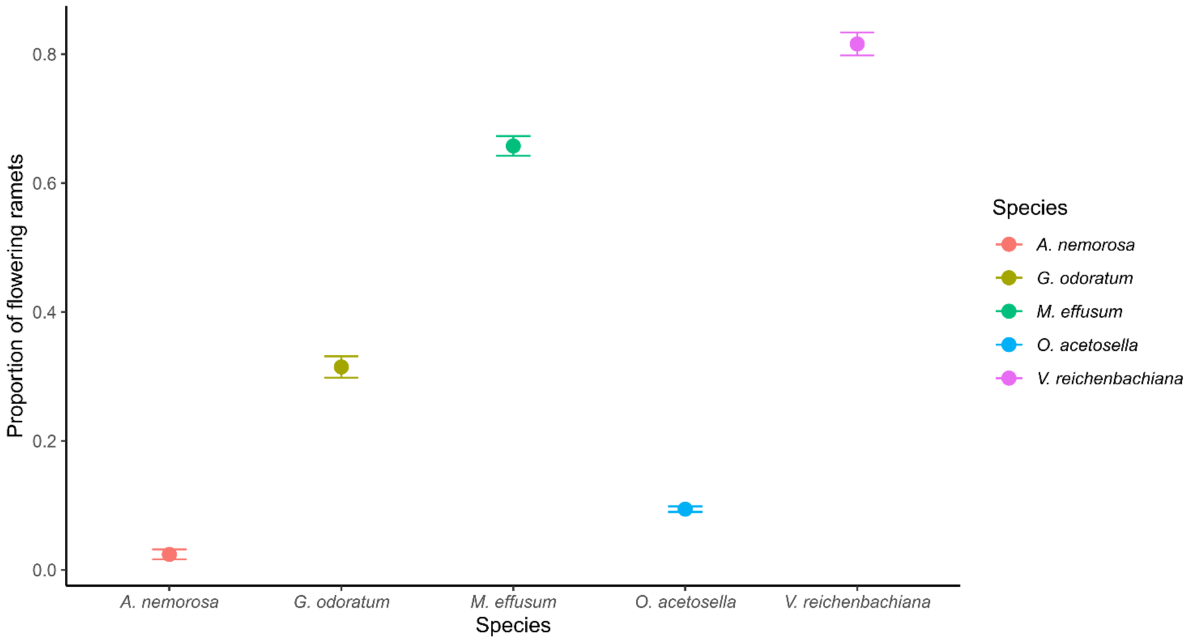

Figure S4. *Proportion of flowering for five species of understory herbs: Anemone nemorosa, Galium odoratum, Milium effusum, Oxalis acetosella and Viola reichenbachiana.*

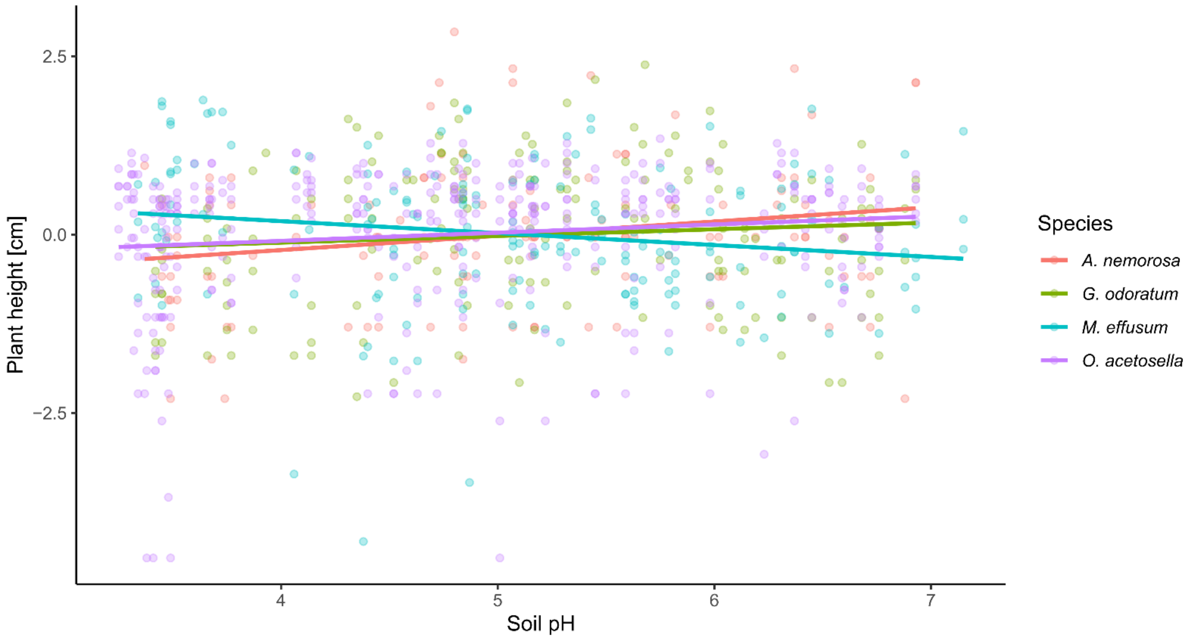

Figure S5. *Linear regressions between plant height and soil pH from the sites of origin for four species of understorey herbs: Anemone nemorosa, Galium odoratum, Milium effusum, and Oxalis acetosella.*

Table S1. *Distribution of sampled individuals among populations of the species Anemone nemorosa, Galium odoratum, Milium effusum, Oxalis acetosella, and Viola reichenbachiana, used for the final measurements. Swabian Alb = AEW, Hainich-Dün = HEW, Schorfheide-Chorin = SEW.*

	Number of individuals				
Population ID	*A. nemorosa*	*G. odoratum*	*M. effusum*	*O. acetosella*	*V. reichenbachiana*
AEW01			3	6	6
AEW02	3	3	4	4	6
AEW03	1	2	5	5	4
AEW04		6	2	5	5
AEW05	3	1	1	5	3
AEW06	4		2	5	5
AEW07				6	4
AEW08	3			4	
AEW09			5		6
AEW10		1	3	6	6
AEW11		4	3	5	6
AEW12		1	5	5	5
AEW13	2	1		5	4
AEW14	3	1	4	4	8
AEW15		1	2	5	2
AEW16	3	1	2		6
AEW17	3	2	3	5	1
AEW18	3	1		6	3
AEW19	1	1	3		2
AEW20	2		1	4	4
AEW21			4		4
AEW22		3	1	4	2
AEW23	2	2			4
AEW24		1	4		
AEW25	2	1			
AEW26		3	2	4	3
AEW27		1	4	5	4
AEW28	1	1			5
AEW29	1		5	5	4
AEW30	1	2	4	6	4
AEW31			3	3	5
AEW32	2	2	4	6	2
AEW33	1	1	4	4	2
AEW34	2		4	6	6
AEW35	1		3		5
AEW36	3	3	5	5	4
AEW37		2	2	5	2
AEW38	1	2	3		4
AEW39	4	3	5	5	3
AEW40	2	3	1	6	4
AEW41		3	3	6	6

AEW42	1	2	4	5	2
AEW43	3	1	4	5	4
AEW44	2	4	1	6	
AEW45	1		2	6	2
AEW46	2	2	2	5	2
AEW47	1	3	3		4
AEW48		1	3		3
AEW49	2	1			3
AEW50		2			
HEW01			1	5	6
HEW03				5	
HEW04		4			
HEW05		3		5	4
HEW06		3		6	
HEW07	1	4		6	
HEW08		3			
HEW09	1	1	2	5	
HEW10	1				
HEW11			1		
HEW13				3	
HEW14		2			
HEW16			2		
HEW17	1	4			
HEW18	2				1
HEW19		1	1		
HEW20	1	5		5	2
HEW21	1	1		3	
HEW22	1	4		6	4
HEW23	1	1		6	5
HEW25	2	3	1		5
HEW26	1	4			
HEW27	2	5			3
HEW28	1	2			
HEW29				4	
HEW30		1	3		
HEW31			2		
HEW32		1			
HEW33	4	5		6	3
HEW34	4				
HEW35	1		2		
HEW36		1	1		
HEW37	1				
HEW38	2				
HEW40	1		3		
HEW41	1				
HEW43	4	3			3
HEW44		2	2		5
HEW45			3		
HEW47	2	3	2		
HEW48		3	1		

HEW49	1	2		6	
HEW51		3		5	6
SEW01					6
SEW02				4	4
SEW03				5	
SEW04				3	
SEW05					6
SEW06	1		2		4
SEW07	1	5			4
SEW08	1				
SEW09	3				
SEW13				6	
SEW14				4	
SEW15					5
SEW16			1	4	5
SEW18				6	
SEW21				4	
SEW22					
SEW23	2			3	
SEW24		5			5
SEW25					6
SEW26				5	5
SEW27			1		
SEW28	3		3	5	6
SEW29				6	
SEW30				6	
SEW31				6	
SEW35			2		5
SEW38				4	
SEW39			2	4	
SEW40	3	1	1	6	6
SEW43	2				
SEW44	1				
SEW46	1		5	5	
SEW47	2	6	4	5	
SEW48	1				
SEW49			1	6	5
SEW50	2		1	6	

TOTAL	**126**	**166**	**178**	**361**	**298**

CHAPTER 2

Forest understorey herbs show adaptation to a regional mosaic of forest management intensities

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SUMMARY

1. Local adaptation plays a fundamental role in the maintenance of genetic diversity. Given the strong effect of forest management on the microenvironment, it seems likely that understorey herbs need to adapt locally. Yet, we know little about local adaptation of understorey herbs to forest management.
2. We tested for local adaptation of *Anemone nemorosa* and *Milium effusum* to forest management using a reciprocal transplant experiment along a management intensity gradient. Genets were sampled from sites with contrasting management intensities and transplanted sympatrically, near and far allopatrically, and to sites where the species was absent. We measured various performance traits over two growing seasons.
3. We found that both local adaptation and phenotypic plasticity help plants to adjust along management and environmental gradients. We observed contrasting patterns of performance between the two species for individuals transplanted to sites where they did not naturally occur, possibly explained by their contrasting dispersal rates.
4. Our study indicates that two understorey herbs with contrasting dispersal abilities vary in their strength of local adaptation and phenotypic plasticity to variation in the understorey environment. Our results underpin the importance of forest management as driver of evolution and for the maintenance of genetic diversity.

Keywords: *Anemone nemorosa*, microclimate, microenvironment, *Milium effusum*, phenotypic plasticity, reciprocal transplant.

INTRODUCTION

Adaptation to local environmental conditions plays a fundamental role in the maintenance of genetic diversity (Blanquart *et al.*, 2013), which is being jeopardized due to land-use transformations (Exposito-Alonso, 2023). Whereas adaptation of plant populations to their local environment has been demonstrated in numerous studies (Leimu & Fischer, 2008; Hereford, 2009; Pluess *et al.*, 2011; Ågren & Schemske, 2012; Bennington *et al.*, 2012; De Frenne *et al.*, 2012; Meineri *et al.*, 2013; De Frenne *et al.*, 2014; Frei *et al.*, 2014), to our knowledge, local adaptation of understorey herbs to forest management remains unexplored to date, despite the common occurrence of forest habitat worldwide.

Forest management actions affect forest structural attributes directly by altering tree species composition, crown projection area, and structural complexity, ultimately resulting in strong effects on the biotic and abiotic forest understorey environment including the microclimate (Willems *et al.*, 2021; Møller *et al.*, 2023a). Forest structural attributes such as canopy openness and structural complexity dictate the local light regime but can also cause temperature buffering effects (De Frenne *et al.*, 2021). Additionally, below-ground environmental factors such as soil pH are strongly affected by dominant tree species, which in turn is often determined by management (Møller *et al.*, 2023a). The understorey environment can, therefore, be described as an environment with strong management-imposed constraints.

Understorey herbs play a key role in the forest ecosystem (Gilliam, 2007; Landuyt *et al.*, 2019), as they harbour the highest biodiversity of all the forest strata and provide important ecosystem services (Gilliam, 2007). Understorey herbs are crucial for nutrient cycling of the forest, and they also contribute to the net primary productivity and annual litter fall (Gilliam, 2007). Previous studies have shown that understorey herbs can be affected greatly by forest structural attributes, not only in species composition and diversity (Valdés *et al.*, 2015), but also in phenology (Willems *et al.*, 2021), and in the genetic basis of ecologically important phenotypic traits (Møller *et al.*, 2023a). Due to the strong effect of management on the forest understorey environment, we can furthermore expect that forest understorey herbs are locally adapted to forest management and management-induced environmental variation.

Local adaptation is a result of divergent selection and evolution, equipping local populations with a fitness advantage under their local environmental conditions compared to foreign environments (Kawecki & Ebert, 2004). Thus, resident genotypes should perform better in their local habitat than genotypes originating from other habitats (Kawecki & Ebert, 2004). One of the prerequisites for local adaptation is relatively low gene flow. We therefore can expect stronger adaptation to local conditions in self-compatible or clonal species compared to outcrossing species (Leimu & Fischer, 2008). An individual's first response to forest management, environmental change, or novel environments, is a plastic response that can be followed by genetic change in the population over the course of generations (Radersma *et al.*, 2020). This indicates that local adaptation involves fine-tuning of environmentally induced phenotypes, causing plasticity to "take the lead" in adaptive evolution (Radersma *et al.*, 2020).

The "gold standard" to study local adaptation is through reciprocal transplant experiments (Gibson *et al.*, 2016; Johnson *et al.*, 2022), as it allows to separate environmental and source of origin effects, and to investigate any interactions between them (i.e., Genotype × Environment). One way to study local adaptation is by 'Sympatric & Allopatric' comparisons (where 'Sympatric' means individuals where origin and transplant site match, and 'Allopatric' means individuals for which their origin and transplant site do not match), allowing the strength of local adaption to be investigated while taking both habitat and population differences into account (Blanquart *et al.*, 2013). As an extension to simple site and population comparisons, management or environmental distances between sites can be considered, e.g. by separating the allopatric component into different degrees of dissimilarity to sympatric combinations (Adiba *et al.*, 2010). The implementation of various degrees of allopatric transplantations also opens up possibilities to investigate the effects of particular environmental factors, rendering this specific approach to investigate local adaptation particularly compelling.

Forest understorey herbs are generally dispersal limited, and species are often absent in forest patches due to either dispersal or establishment limitations (Hermy & Verheyen, 2007). Previous studies have confirmed that phenotypic variation and their genetic and plastic components play important roles in the adaptation of plants to future climatic conditions (Møller *et al.*, 2023b). The transplantation of individuals into novel environments in combination with investigations of phenotypic variation and plant performance after transplantation could provide useful insights in dispersal and establishment limitations (Spiecker, 2003). Furthermore, by adding environmental data, the degree of novelty of a foreign habitat to focal species can be confirmed and transplantation experiments can be used to test for pre-adaptation of species to future conditions, which can be valuable information for ecological restoration (Ehrlén & Eriksson, 2000).

Here we apply a reciprocal transplant experiment to assess whether populations of two common European forest understorey herbs show local adaptation to forest management and to local environmental variables. Furthermore, we investigate if the strength of local adaptation to forest management and to environmental variables varies between the species. And finally, we explored if unoccupied sites are a result of establishment limitations or of limited dispersal abilities of the species.

MATERIALS AND METHODS

Study species

We used two plant species representative of the understorey herb community, *Anemone nemorosa* (Ranunculaceae) and *Milium effusum* (Poaceae). The two study species are common and occur throughout German temperate forests (De Frenne *et al.*, 2010; De Frenne *et al.*, 2017).

Anemone nemorosa is an early-flowering forb, relying heavily on clonal spread through rhizomes (Frederiksen & Rasmussen, 2006). Despite having a low dispersal rate and gene flow (Brunet *et al.*, 2012), sexual reproduction is still considered important for population persistence (Frederiksen & Rasmussen, 2006). *Anemone nemorosa* is a shade tolerant species, growing primarily in temperate woodland conditions where its yearly reproductive cycle is suited to the leaf-out dynamics of the forest (Shirreffs, 1985b).

Milium effusum is a tall-growing tussock grass relying on wind-dispersed seeds after flowering in the summer, but re-sprouting through short stolons is also used for colonization (De Frenne *et al.*, 2011a). *Milium effusum* is a shade tolerant species, growing typically in temperate deciduous forests, but it can also occur along railways and roads (De Frenne *et al.*, 2017).

Experimental design

We conducted a reciprocal transplant experiment on a total of 39 plots in all three regions of the Biodiversity Exploratories (www.biodiversity-exploratories.de) distributed across Germany: Schwäbische-Alb, Hainich-Dün, and Schorfheide-Chorin (Fischer *et al.*, 2010). Each region contained 50 forest plots of 100 × 100 m, ranging from protected and natural areas to highly managed timber plantations. In order to cover the whole gradient of forest management intensity (abbreviated as SMI, i.e., silvicultural management intensity; Schall and Ammer, 2013) within each region, we chose our plots of origin by ranking all 50 plots from each region from low to high management intensity. We then divided the gradient of each region into low, mid, and high management intensity bins and, per species, chose three plots from each bin. We also assigned three plots per region where the focal species did not occur ('Absent'), one plot per bin. We subsequently assigned three groups of plots per region such that each group contained one origin plot from each bin and one additional 'Absent' plot. In spring 2020, we sampled six genets from each origin plot, divided each genet into four cuttings and potted all cuttings into multitrays (5 cm ø and 5.5 cm deep, 54 pots per tray). A cutting of *A. nemorosa* was a piece of rhizome ca. 4 cm long buried in the soil whereas a cutting of *M. effusum* consisted of a single shoot with roots. The multitrays were filled with potting soil (CL T torffrei, Einheitserde, Sinntal-Altengronau, Germany) and were placed outdoors in Tübingen and, from September 2020, in Frankfurt am Main, Germany.

In October 2020, we brought the cuttings back to their respective regions and transplanted them sympatrically to their home plot of origin ('Sympatric'), allopatrically to the remaining two plots of origin for the cutting's group ('Allopatric'), as well as to the 'Absent' plot (see Figure S1). A cutting originating from an intermediate intensity plot would be allopatrically transplanted to two 'Near allopatric' plots, one in the low and one in the high intensity plot, whereas a cutting originating from a low or high intensity plot would be allopatrically transplanted to one 'Near allopatric' and one 'Far allopatric' plot (Figure 1). On each plot the cuttings were transplanted within a fenced area to prevent damage by large animals. Each cutting was taken out of the multitray, planted into a 1.5 L pot filled with local soil from the plot, and the pot was dug into the soil such that the top of the pot was at ground level (Figure S1). In total, we transplanted 648 *A. nemorosa* cuttings (3 regions × 9 origins × 6 genets × 4 cuttings). However, due to low abundance of *M. effusum* in Schorfheide-Chorin, we could only sample and reciprocally transplant this species in the two other regions, adding up to 432 *M. effusum* cuttings (2 regions × 9 origins × 6 genets × 4 cuttings).

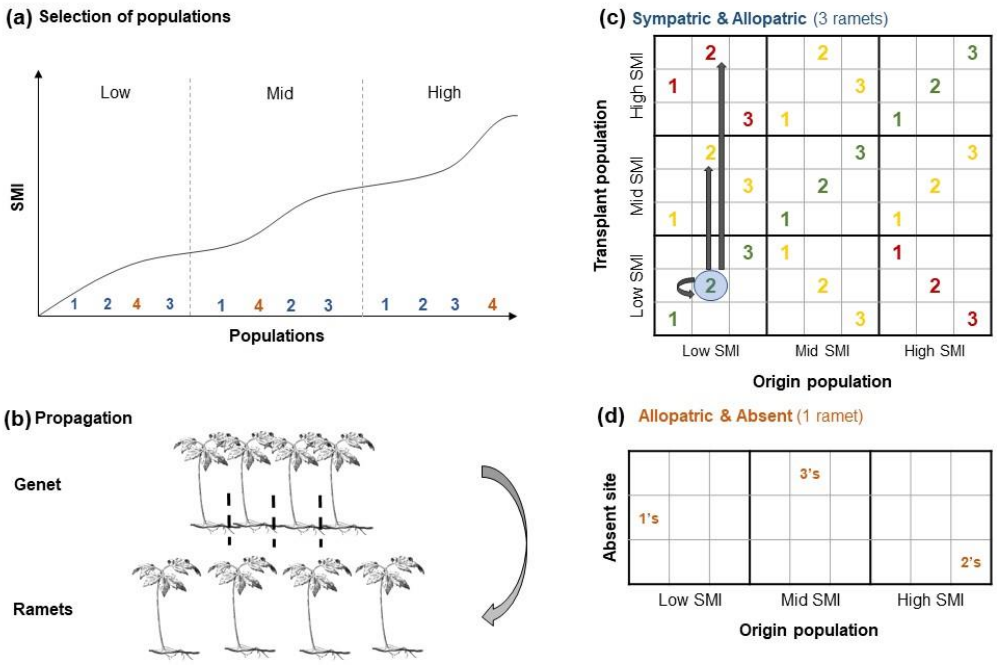

***Figure 1**: **Schematic of the experimental design.** (**a**) For each region, all 50 plots were ranked from low to high forest management intensity (SMI). We then created equally broad bins (in terms of SMI distance) for low, mid, and high SMI populations, and we chose populations of origin from each bin (restricted to plots we knew one or both species occurred), replicated three times (1, 2 & 3). (**b**) We sampled six genets from each population and propagated each genet into four cuttings. (**c**) 'Sympatric & Allopatric' comparisons were conducted for each replicate population (1, 2 & 3) by transplanting cuttings of all genets sympatrically (green), near allopatrically (yellow), and far allopatrically (red). (**d**) 'Sympatric & Absent' comparisons were conducted for each replicate population by choosing a site (4 in panel a) from each bin (low, mid & high) where the species did not naturally occur and to which all replicates (1's, 2's or 3's) were transplanted.*

Measured traits

Plant measurements were taken in 2021 and 2022. Each year, measurements for *A. nemorosa* were taken in April, which is flowering period for this species, and measurements for *M. effusum* in June, when this species flowers. We considered each vegetative or reproductive shoot of *A. nemorosa* as well as each shoot of *M. effusum* as a single "ramet". For each pot, we measured plant height to the nearest 0.5 cm on the tallest ramet, we counted the total number of ramets, and we ranked the phenological stage for each ramet (0 = no plant visible, 1 = emerging ramet, 2 =

vegetative ramet, 3 = ramet with flowering bud, 4 = ramet currently flowering or done flowering). We then calculated the performance value for each individual (i.e., each pot) as the sum of the rankings. Furthermore, in 2022, we collected the total aboveground biomass for each pot and dried it in a drying oven at 65 °C for 48 hours before we weighed it.

Environmental variables

As explanatory environmental variables, we considered SMI, structural complexity of the forest stand, soil pH and the mean annual spring temperature. The chosen environmental variables are known to be strongly affected by forest management and forest structural attributes (Willems *et al.*, 2021; Møller *et al.*, 2023a). All environmental variables were extracted for each plot for both 2021 and 2022 from the BExIS2 (www.bexis.uni-jena.de) database, maintained by the Biodiversity Exploratories (Fischer *et al.*, 2010). The environmental difference (Δ) was calculated as the difference from each plant's plot of origin to that plant's plot of transplantation. Furthermore, soil moisture count and temperature at +15 cm, 0 cm, and -8 cm relatively to the soil surface was measured every 15 minutes for the whole duration of the experiment using TOMST TMS4 loggers, deployed among the transplanted plots (Wild *et al.*, 2019).

Data analyses

All statistical analyses were conducted with R version 4.1.2 (R Core Team, 2021a). First, mortality, referring to the absence of any ramets in a pot, was calculated for both years for each species within each transplant category. We ran a generalized linear model using the function 'glm' (R Core Team, 2021a). Mortality was set as the response variable (binomial), and year, species, transplant category ('Sympatric', 'Near allopatric', 'Far allopatric', and 'Absent'), as well as their two- and three-way interactions were set as the explanatory variables.

Second, we pooled the near and far allopatric sites together and hereafter call them 'Allopatric'. We tested for differences in 'Sympatric & Allopatric' and 'Sympatric & Absent' transplants separately. For this we ran linear mixed-effects models (LMMs) for all traits in a total of eight separate models, using the function 'lmer' from the package 'lme4' (Bates *et al.*, 2007). The measured traits were used as response variables, and the transplant categories (either 'Sympatric' and 'Allopatric' or

‘Sympatric’ and ‘Absent’) were used as the explanatory variables. Genet, population, and region were set as independent random factors.

Third, we tested for the effects of environmental differences (∧) among the origin and transplant plots. We ran LMMs for all traits using the function ‘lmer’. The measured traits were used as the response variables, and the linear and quadratic terms of ΔSMI, Δstructural complexity, Δsoil pH, and Δyearly mean spring temperature between origin and transplant plots were included as the explanatory variables. Genet, population, and region were included as independent random factors. To assess the robustness of the linear and quadratic terms, a generalized additive model (GAM) was fitted on the regression plots to check the overlap with the linear and quadratic regression fits. Due to the scarcity of significant results in 2021 compared to 2022 (Table S3), possibly due to plants not having accumulated significant differences in their performance, we only investigated the effects of the environmental variables for 2022.

All model test results were obtained by applying the function ‘Anova’ from the package ‘car’ (Fox *et al.*, 2012). Shapiro-Wilk and Bartlett tests were used to assess whether assumptions of normality and homoscedasticity of the model residuals were met, respectively. When at least one of the assumptions was violated, the response variable was transformed and used if it improved the distribution of residuals.

RESULTS

Mortality was documented first in 2021, when populations had > 45% losses in both species in the ‘Absent’ sites, while lower percentages of mortality were observed in the ‘Sympatric’ and ‘Allopatric’ sites (8-21%; Fig. **2**). In 2022, additional mortality occurred in the ‘Absent’ sites for both species (5-13% of the initial transplants). Compared to 2021, for ‘Sympatric’ and ‘Allopatric’ sites in 2022 *A. nemorosa* experienced a low additional mortality (10-11%), whereas the additional mortality in 2022 was markedly higher in *M. effusum* for ‘Sympatric’ and ‘Allopatric’ sites (27-31%) (Fig. **2**). However, due to large variation in mortality among transplant plots, mortality did not differ significantly among categories, years, or species (Table **S2**).

Tests for population differences between transplant categories ‘Sympatric & Allopatric’ revealed no significant differences in the measured traits from either species in 2021 or 2022 (Table **1**). In 2021, ‘Sympatric & Absent’ comparisons showed significant differences between the categories only for performance value in *A.*

nemorosa. In 2022, all measured traits differed significantly between ‘Sympatric vs Absent’ for both species, with stronger effects in *A. nemorosa* than in *M. effusum* (Table **1**). Moreover, the species showed opposing patterns in all measured traits: *A. nemorosa* populations performed better as ‘Sympatric’ sites, whereas *M. effusum* populations performed better at ‘Absent’ sites (Fig. **3**).

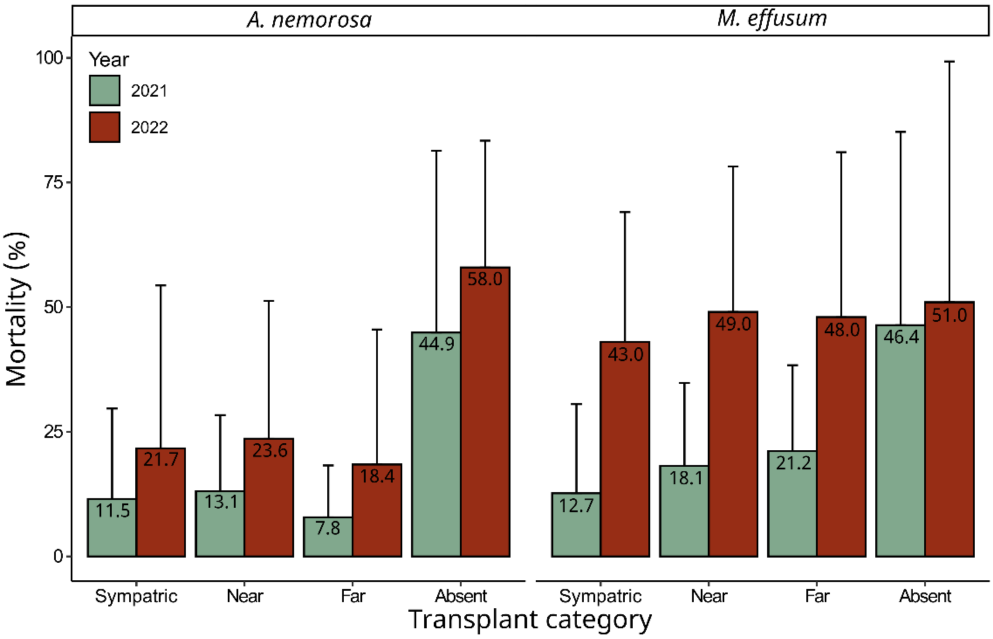

Figure 2. The percentage mortality for the different transplant categories Sympatric, Near allopatric, Far allopatric and Absent for both years (compared to the start of the experiment) for the study species Anemone nemorosa and Milium effusum. *Numbers within the bars indicate the mean across populations. The error bars indicate the standard error across populations.*

Next, we tested for effects of environmental differences (Δ) between population origins and transplant sites on the measured traits in 2022. In ‘Sympatric & Allopatric’ models for *A. nemorosa*, the linear effect of ΔSMI was significant in all measured traits (Table **2**), showing an increase in the measured traits when transplanted to sites with lower SMI (Fig. **4a**, **5b** **6a**, and **7b**). Similarly, the linear effect of ΔSpring temperature was significant in all measured traits for *A. nemorosa* (Table **2**), showing an increase in traits when transplanted to colder sites (Fig. **4d**, **5d**, **6c**, and **7a**). Furthermore, the quadratic effect of ΔStructural complexity was significant in all measured traits for *A. nemorosa* (Table 2), showing the highest fitness at sympatric sites (Fig. **4b** **5a**, **6b**, and **7d**).

Table 1. Results of mixed-effects models of the measured traits in Anemone nemorosa and Milium effusum for 2021 and 2022 as response variables, and the transplant categories (i.e., Sympatric & Allopatric, or Sympatric & Absent) as the explanatory variables. *Population, genet, and region were set as random factors. Significant P-values are indicated in bold followed by * P < 0.05; ** P < 0.01; *** P < 0.001.*

	Anemone nemorosa				*Milium effusum*			
	2021		**2022**		**2021**		**2022**	
	χ^2	P-value	χ^2	P-value	χ^2	P-value	χ^2	P-value
Response variable	**Sympatric & Allopatric**							
Plant height	2.127	0.345	2.448	0.294	1.883	0.390	0.738	0.691
Number of ramets	0.705	0.703	1.646	0.439	0.592	0.744	0.034	0.983
Performance score	1.973	0.373	1.374	0.503	2.537	0.281	0.372	0.830
Biomass			5.569	0.062			0.026	0.987
Response variable	**Sympatric & Absent**							
Plant height	2.434	0.127	28.51	**<0.001 *****	2.117	0.146	5.532	**0.019 ***
Number of ramets	3.299	0.069	27.95	**<0.001 *****	2.760	0.097	4.738	**0.030 ***
Performance score	24.51	**<0.001 *****	32.61	**<0.001 *****	2.695	0.101	6.762	**0.009 ****
Biomass			42.29	**<0.001 *****			20.32	**<0.001 *****

Table 2. Results of mixed-effects models using performance traits in A. nemorosa and M. effusum for 2022 at 'Sympatric & Allopatric' and 'Sympatric & Absent' transplantation sites as response variables, and the linear and quadratic terms of environmental differences (Δ) between the site of origin and the site of transplantation as fixed factors. *As response variables we tested plant height, number of ramets, performance score and biomass, and as environmental variables we included structural management intensity (SMI), structural complexity, soil pH and mean annual spring temperature. Population, genet, and region were set as random factors. Significant P-values are indicated in bold followed by * P < 0.05; ** P < 0.01; *** P < 0.001.*

		Anemone nemorosa				*Milium effusum*			
		Sympatric & Allopatric		**Sympatric & Absent**		**Sympatric & Allopatric**		**Sympatric & Absent**	
		χ^2	P-value	χ^2	P-value	χ^2	P-value	χ^2	P-value
Response variable	**Explanatory variable**								
Plant height	ΔSMI	35.62	**<0.001 *****	1.787	0.181	0.417	0.493	0.046	0.830
	ΔSMI^2	3.820	0.051	1.247	0.264	1.117	0.291	1.082	0.298
	Δstructural complexity	9.150	**0.002 ****	17.13	**<0.001 *****	0.030	0.862	1.340	0.247
	Δstructural complexity^2	6.451	**0.011 ***	0.741	0.390	0.360	0.548	1.573	0.210
	Δsoil pH	0.525	0.469	0.454	0.500	38.10	**<0.001 *****	3.625	0.057
	Δsoil pH^2	0.000	0.997	1.084	0.298	2.875	0.090	4.060	**0.044 ***
	Δspring temperature	40.92	**<0.001 *****	0.022	0.882	0.745	0.388	0.006	0.937
	Δspring temperature^2	3.065	0.080	0.017	0.897	0.348	0.555	1.068	0.301
Number of ramets	ΔSMI	20.40	**<0.001 *****	2.751	0.097	0.430	0.512	0.126	0.723
	ΔSMI^2	0.907	0.341	1.349	0.245	0.063	0.803	0.001	0.973
	Δstructural complexity	0.126	0.722	0.058	0.810	0.217	0.641	0.402	0.526
	Δstructural complexity^2	8.741	**0.003 ****	0.439	0.508	4.116	**0.042 ***	0.422	0.516
	Δsoil pH	19.21	**<0.001 *****	4.470	**0.034 ***	5.391	**0.020 ***	1.902	0.168
	Δsoil pH^2	0.001	0.981	0.101	0.750	0.286	0.593	2.757	0.097

	Δspring temperature	15.19	**<0.001 *****	0.180	0.671	1.442	0.230	0.165	0.684
	Δspring temperature^2	1.928	0.165	1.290	0.356	0.158	0.691	0.200	0.655
Performance score	ΔSMI	24.33	**<0.001 *****	2.148	0.143	0.734	0.392	0.006	0.937
	ΔSMI^2	0.675	0.411	0.837	0.360	0.003	0.954	0.178	0.673
	Δstructural complexity	0.509	0.475	0.001	0.983	2.121	0.145	1.234	0.267
	Δstructural complexity^2	10.37	**0.001 ****	0.789	0.375	1.869	0.172	0.486	0.486
	Δsoil pH	20.64	**<0.001 *****	4.722	**0.030 ***	12.46	**<0.001 *****	2.172	0.141
	Δsoil pH^2	0.056	0.813	0.197	0.657	0.024	0.876	2.520	0.112
	Δspring temperature	15.18	**<0.001 *****	0.579	0.447	0.108	0.743	0.389	0.533
	Δspring temperature^2	1.599	0.206	0.946	0.331	0.171	0.680	0.427	0.513
Biomass	ΔSMI	48.24	**<0.001 *****	1.684	0.194	0.275	0.600	0.041	0.841
	ΔSMI^2	4.636	**0.031 ***	0.242	0.623	0.363	0.547	0.395	0.530
	Δstructural complexity	2.170	0.141	0.483	0.487	0.126	0.723	1.218	0.270
	Δstructural complexity^2	8.844	**0.003 ****	2.838	0.092	1.288	0.256	0.407	0.523
	Δsoil pH	26.26	**<0.001 *****	4.288	**0.038 ***	41.90	**<0.001 *****	1.469	0.226
	Δsoil pH^2	0.330	0.565	0.004	0.905	1.281	0.258	2.043	0.153
	Δspring temperature	10.11	**0.001 ****	3.307	0.069	3.938	**0.047 ***	0.081	0.776
	Δspring temperature^2	1.919	0.166	2.853	0.091	0.750	0.387	0.671	0.413

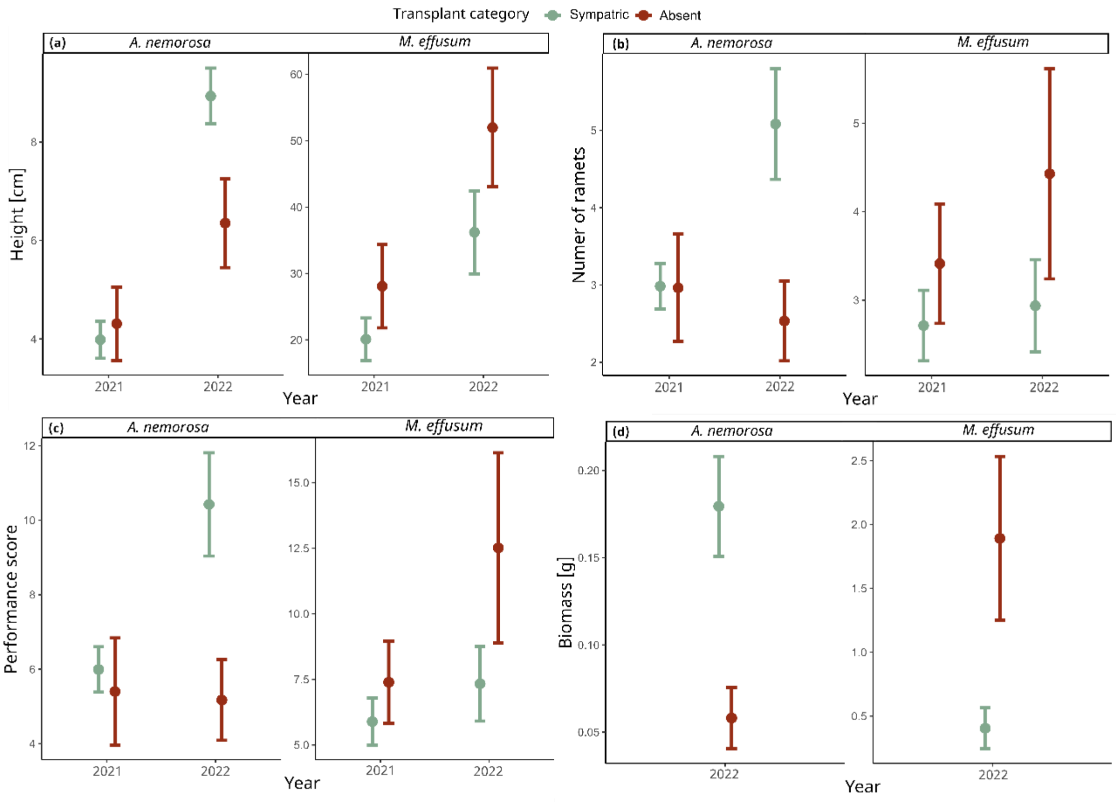

Figure 3: Plant performance in 2021 and 2022 for the 'Sympatric' and 'Absent' transplantation sites for Anemone nemorosa and Milium effusum. (a) *plant height,* ***(b)*** *number of ramets,* ***(c)*** *performance score, and* ***(d)*** *aboveground biomass. Circles indicate means across populations, and the error bars indicate the standard error.*

We also found that for *A. nemorosa* plant height increased when transplanted to populations in more structurally complex forests (Fig. **4b**). The linear effect of ΔSoil pH was significant for plant height, number of ramets, performance value, and biomass (Table **2**), all traits showing an increase in individuals transplanted to less acidic soils (Fig. **4c**, **5c**, **6d**, and **7c**). And lastly for *A. nemorosa*, we see a significant relationship between quadratic ΔSMI and biomass, with higher biomass at sympatric sites (Table **2**, Fig. **7b**). In the 'Sympatric & Allopatric' models for *M. effusum*, the linear effect of ΔSoil pH was significant for all measured traits (Table **2**; Fig. **4c**, **5c**, **6d**, **7c**, with increasing values in individuals transplanted to less acidic sites. Furthermore, we found a significant relationship between quadratic ΔStructural complexity and the number of ramets (Table **2**), where this is a convex relationship with individuals of *M. effusum* having less ramets at sympatric sites (Fig. **4a**). Finally, a significant linear relationship between ΔSpring temperature and biomass of *M. effusum* (Table **2**) reflected an increase in biomass in individuals transplanted to colder sites (Fig. **7a**).

For the ‘Sympatric & Absent’ models, we found only few statistically significant relationships. For *A. nemorosa*, number of ramets, performance value and biomass significantly increased with decreasing soil pH (Table **2**; Fig. **8c**, **8d**, **8e**). However, plant height significantly decreased in structurally less complex forest plots (Table **2**, Fig. **8a**). As the only significant relationship in the ‘Sympatric & Absent’ models for *M. effusum*, we observed a convex relationship between ΔSoil pH and plant height (Table **2**; Fig. **8b**).

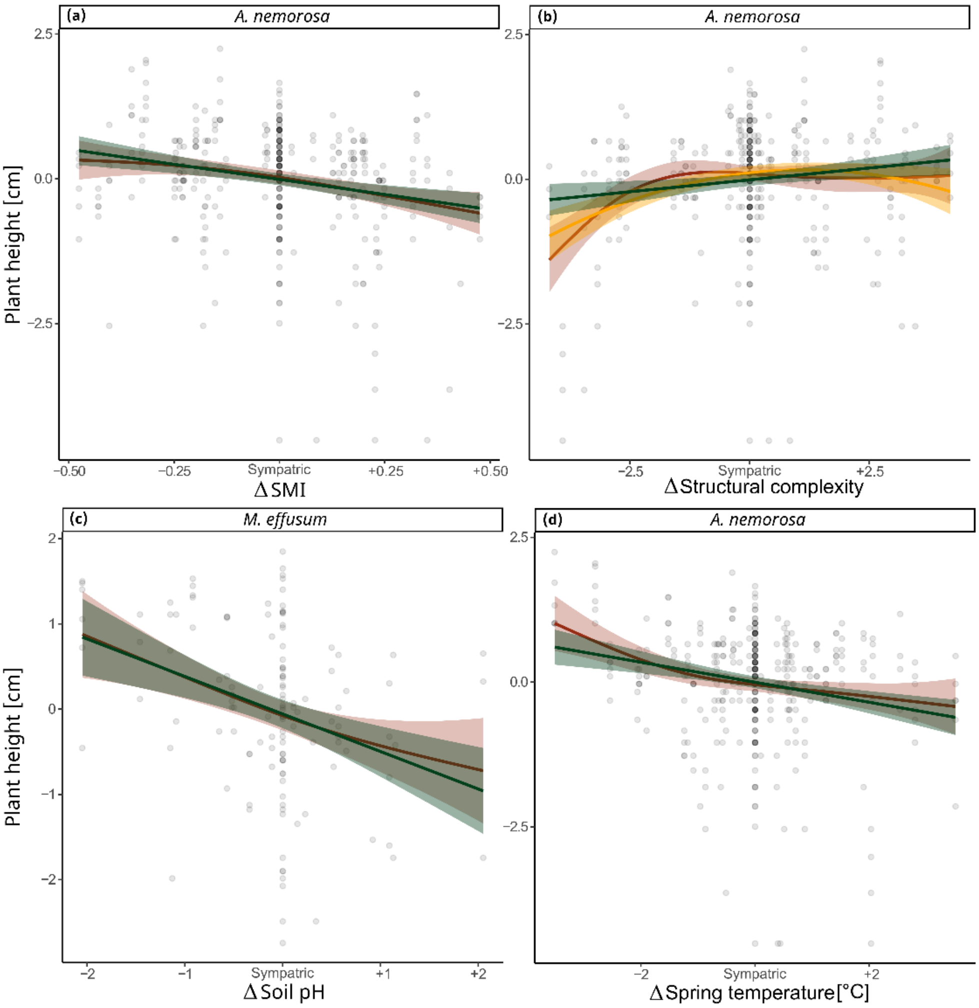

Figure 4: Significant relationships between plant height for ‘Sympatric & Allopatric’ in 2022 and the environmental differences. *Δ denotes the environmental differences between sympatric and transplant sites for* ***(a)*** *forest management intensity (SMI) for Anemone nemorosa,* ***(b)*** *structural complexity for A. nemorosa,* ***(c)*** *soil pH for Milium effusum,* ***(d)*** *spring temperature for A. nemorosa. Linear regression fits are green, quadratic fits are yellow and gam fits are red. 95%*

confidence intervals are shown around the curves. The data points have been scaled for each trait and species separately.

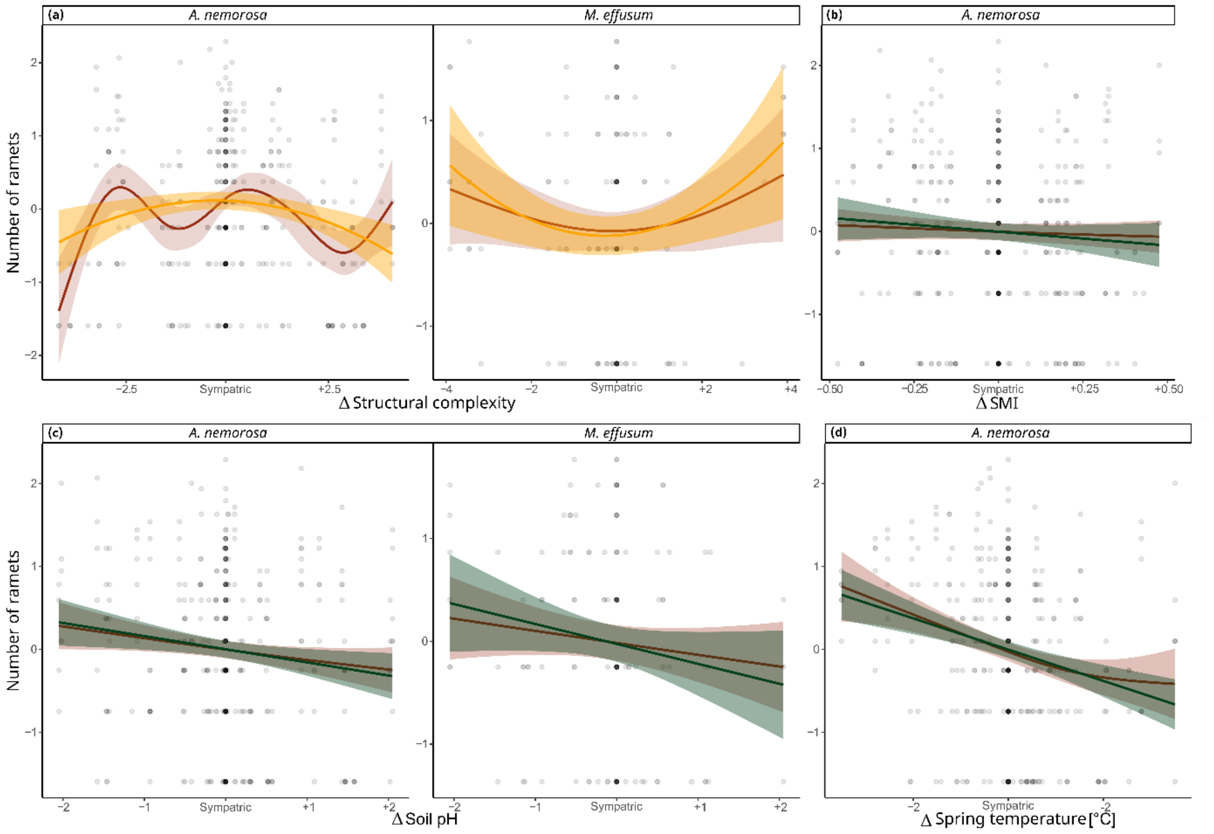

***Figure 5: Significant relationships between number of ramets for 'Sympatric & Allopatric' in 2022 and the environmental differences.** Δ denotes the environmental differences between sympatric and transplant sites for both Anemone nemorosa and Milium effusum, **(b)** forest management intensity (SMI) for A. nemorosa, **(c)** soil pH for A. nemorosa and M. effusum, and **(d)** spring temperature for A. nemorosa. Linear regression fits are green, quadratic fits are yellow and gam fits are red. 95% confidence intervals are shown around the curves. The data points have been scaled for each trait and species separately.*

DISCUSSION

Overall, our study reveals evidence for local adaptation of *A. nemorosa* to forest structural attributes and the forest microenvironment. The evidence consists of higher performance in Sympatric & Absent sites and in few but consistent quadratic relationships between plant performance and the environmental difference with the site of origin. Interestingly, in contrast to *A. nemorosa*, *M. effusum* performed best at Absent sites. Linear relationships between the phenotypic expression of the populations and environmental drivers at the transplant sites showed that phenotypic plasticity plays an important role in shaping plant phenotypes along environmental gradients.

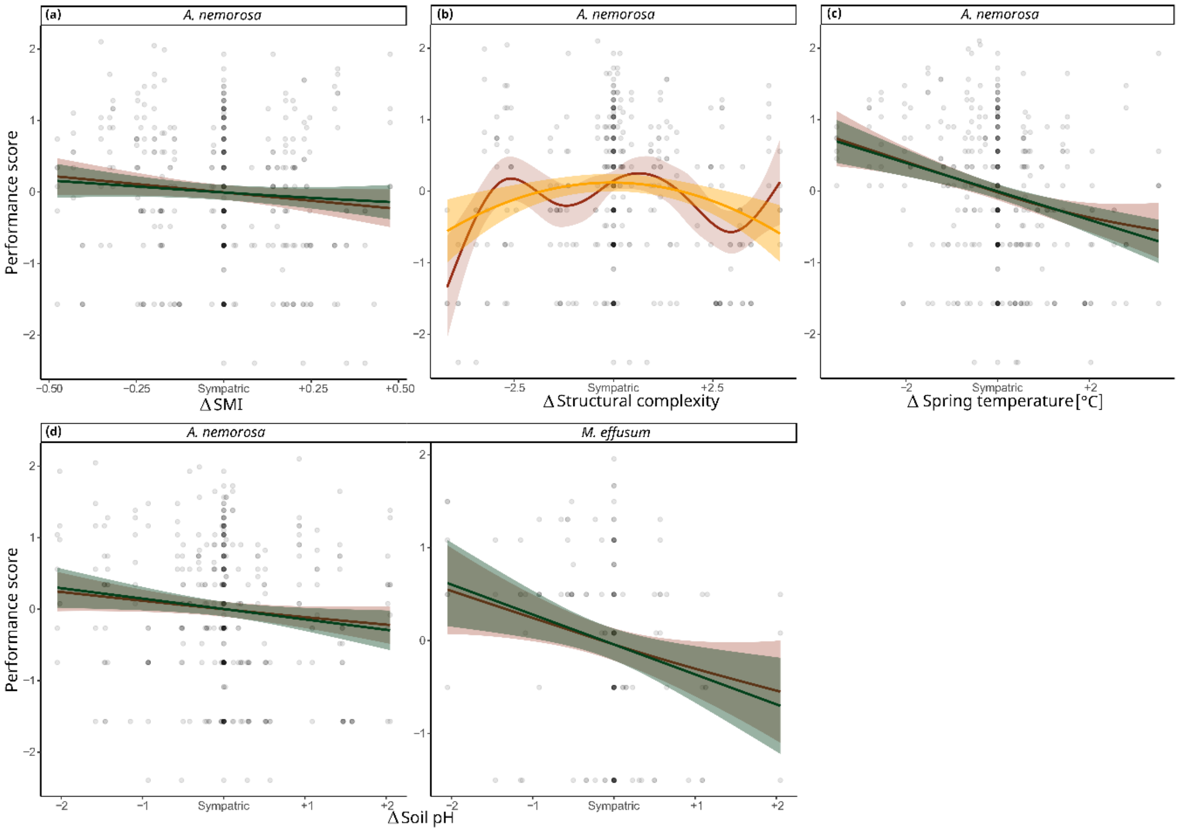

Figure 6: Significant relationships between performance score for 'Sympatric & Allopatric' in 2022 and the environmental differences. *Δ denotes the environmental differences between sympatric and transplant sites for* ***(a)*** *forest management intensity (SMI) for Anemone nemorosa,* ***(b)*** *structural complexity for A. nemorosa,* ***(c)*** *spring temperature for A. nemorosa, and* ***(d)*** *soil pH for both A. nemorosa and Milium effusum. Linear regression fits are green, quadratic fits are yellow and gam fits are red. 95% confidence intervals are shown around the curves. The data points have been scaled for each trait and species separately.*

'Sympatric & Allopatric' comparisons

Differences in mortality between sympatric, near allopatric, and far allopatric sites are statistically negligible in both years for both species, possibly due to the large standard errors caused by strong population differences. Nevertheless, for *A. nemorosa* a small portion of plants died in both 2021 and 2022, whereas for *M. effusum* the percentage of mortality was higher, especially in 2022. In our design, both species shared most of the origin and transplant plots and thus experienced the same environmental conditions in both years, excluding different environments between species as an explanation for the observed differences in mortality. However, the species differ in their life history, which may explain mortality discrepancies. *Anemone nemorosa* is a spring geophyte and thus has dormant rhizomes belowground for most of the year,

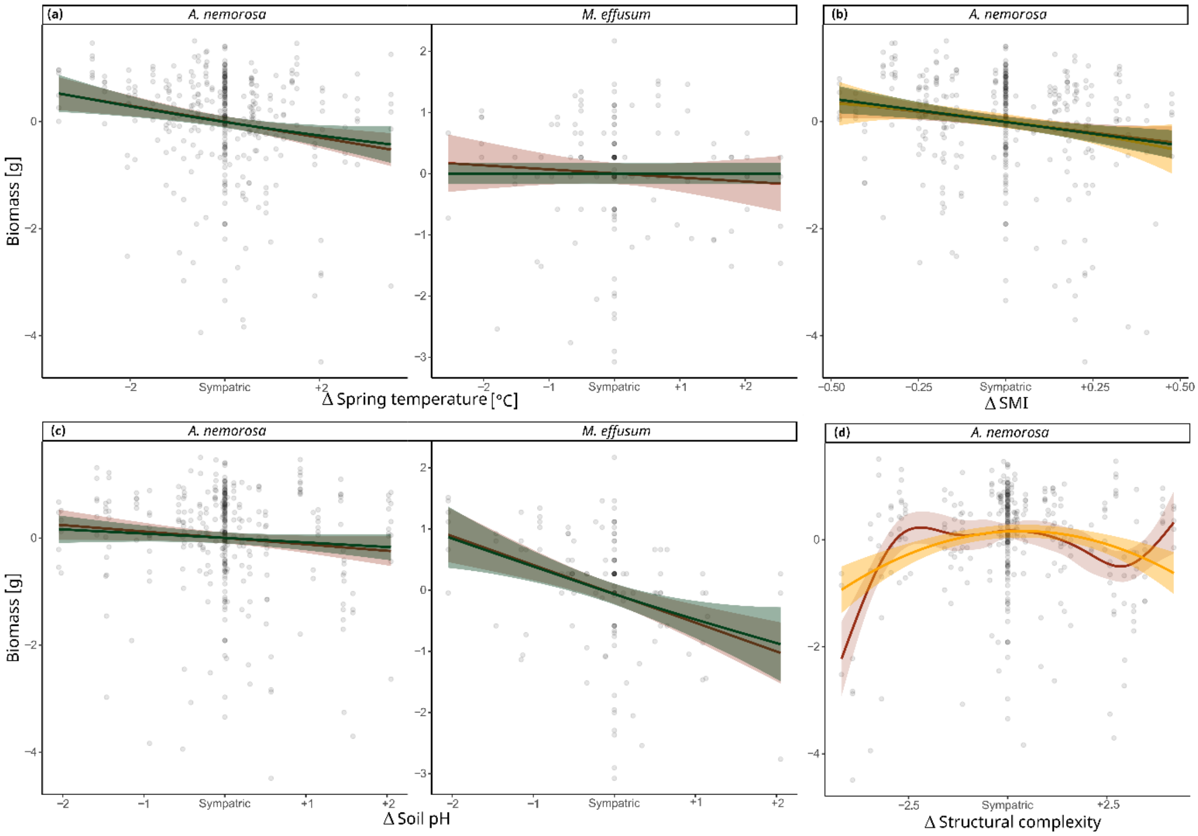

Figure 7: Significant relationships between biomass for 'Sympatric & Allopatric' in 2022 and the environmental differences. *Δ denotes the environmental differences between sympatric and transplant sites for* ***(a)*** *spring temperature for both Anemone nemorosa and Milium effusum,* ***(b)*** *forest management intensity (SMI) for A. nemorosa,* ***(c)*** *soil pH for both A. nemorosa and M. effusum, and* ***(d)*** *structural complexity for A. nemorosa. Linear regression fits are green, quadratic fits are yellow and gam fits are red. 95% confidence intervals are shown around the curves. The data points have been scaled for each trait and species separately.*

well protected from potential droughts. *Milium effusum*, in contrast, is exposed to the aboveground environment all year round, rendering it more vulnerable to, e.g., summer droughts or frost damage, which may explain the higher mortality. In fact, in 2022 lower soil moisture than in 2021 was detected in the critical summer months just before measurements for *M. effusum* were taken (Figure S2).

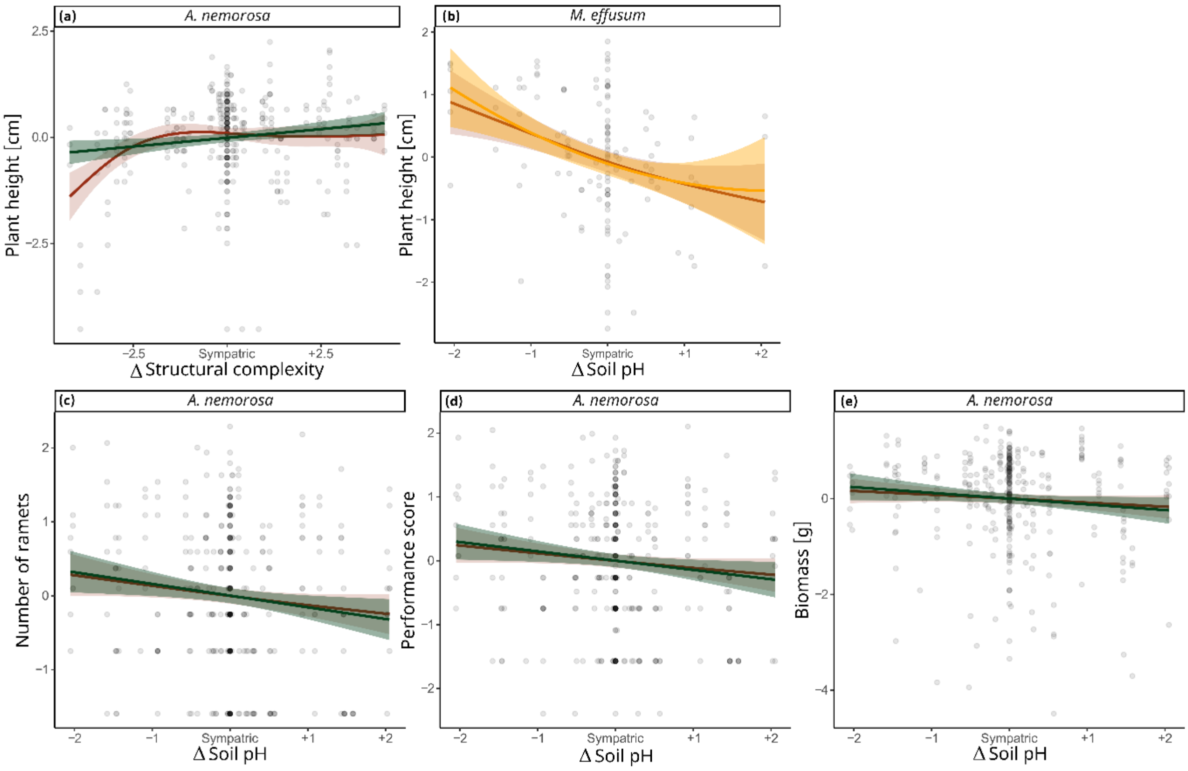

Figure 8: Significant relationships for ‘Sympatric & Absent’ in 2022 and the environmental differences. *Δ denotes the environmental differences between sympatric and absent transplant sites for* ***(a)*** *height and structural complexity for Anemone nemorosa,* ***(b)*** *height and soil pH for Milium effusum,* ***(c)*** *number of ramets and soil pH for both A. nemorosa,* ***(d)*** *performance score and soil pH for A. nemorosa, and* ***(e)*** *biomass and soil pH for A. nemorosa. Linear regression fits are green, quadratic fits are yellow and gam fits are red. 95% confidence intervals are shown around the curves. The data points have been scaled for each trait and species separately.*

Comparisons between ‘Sympatric & Allopatric’ for each trait yielded no differences, potentially due to the implementation of artificial categories of near and far allopatric sites based on the SMI gradient. Indeed, when we switch to a continuous approach and use environmental differences between origin and transplant plots for several environmental variables, many linear and some quadratic relationships between our measured traits and the environmental distance of the plants to their site of origin became apparent. Only few ‘Sympatric & Allopatric’ relationships along the continuous gradient were present in 2021, while many significant relationships were found in 2022. This is likely because the individuals were establishing in their transplanted environment in 2021 and time was still too short to accrue phenotypic differences that reflect adaptation after transplantation. Other studies likewise show that several years can be necessary in order to detect local adaptation (Bennington *et al.*, 2012; Johnson *et al.*, 2022).

In 2022, the significant relationships between the measured traits and the forest structural attributes show the ability of the studied understorey herbs to adapt to environmental features. Most of these relationships were linear, indicating that fitness is not highest at the sympatric sites but rather that phenotypic plasticity shapes phenotypes along environmental gradients. This is in line with the co-gradient variation hypothesis, stating that favourable environmental conditions can cause increased trait expression along an environmental gradient (Conover & Schultz, 1995). All measured *A. nemorosa* traits increased when transplanted to plots with lower SMI, soil pH and spring temperature. A lower forest management intensity can be tightly linked to a higher structural complexity and might promote increased trait performance due to heterogeneity in the forest stand and more light influx. Furthermore, individual seems to benefit from a lower soil pH and sites with colder mean annual spring temperature. In the face of global warming, the optimal thermal niche of *A. nemorosa* might be increasingly more located in colder, coniferous plots, also associated with a lower soil pH. And finally, the consistent quadratic effects of structural complexity, suggest that this specific forest structural attribute (or any environmental factor correlating with it) is an important driver of local adaptation in forest understorey herbs. We even see one significant convex relationship in *M. effusum* between number of ramets and structural complexity, suggesting maladaptation. Considering that we barely see any relationships in the first year of measurements in 2021, but observed considerable indications of phenotypic plasticity and local adaptation in the second year of measurements in 2022, it is within reason to anticipate the effects of local adaptation to become stronger with time (Bennington *et al.*, 2012). The detection of local adaptation within the three investigated regions indicates the evolutionary potential to adapt to a geographic mosaic of structural complexity related to forest management intensity and may suggest that the studied species have been subjected to strong selective forces that overcame gene flow within each region.

Similar transplant experiments where local populations have been transplanted along an environmental gradient have produced a variety of outcomes. Often the local genotype performs best in its sympatric site, indicating strong local adaptation (Ågren & Schemske, 2012; Bennington *et al.*, 2012; De Frenne *et al.*, 2012; Anderson *et al.*, 2013; Toräng *et al.*, 2015). However, significant phenotypic plasticity resulting in increased overall performance under changed environmental conditions at the transplant sites are also common (Pluess *et al.*, 2011; Meineri *et al.*, 2013). Our observation of phenotypic plasticity displayed via significant linear relationships between the majority of traits and environmental differences indicates increased ecological resilience and adaptive potential, as phenotypic plasticity is a necessary building block for local adaptation (Radersma *et al.*, 2020).

'Sympatric & Absent' comparisons

The mortality occurring in 2021 mainly occurred on 'Absent' plots, where the species did not naturally occur. This supports the possibility of establishment limitations, rather than the possibility that these sites are uninhabited due to dispersal limitation in both species (Ehrlén & Eriksson, 2000). The lack of significant relationships between the measured traits and the environmental differences in the 'Sympatric & Absent' models indicates that we can rule out the specific environmental variables included in this study as causing the sites to be unsuitable. Since the fundamental niche of a species is generally broader than the realized niche, further research could consider the role of biotic factors such as herbivory, plant-soil biota interactions or pathogens as explanations for the species' absence on these sites (Nooten & Hughes, 2017).

When comparing the measured performance traits between 'Sympatric & Absent' in 2021, no significant differences were observed for either species, but these effects were striking in 2022. In 2022, *A. nemorosa* individuals always performed better at sympatric sites, strongly indicating local adaptation, while *M. effusum* showed the exact opposite pattern, always performing better when transplanted to sites where they did not naturally occur. In a previous study by De Frenne et al. (2014), an increase in fitness in *M. effusum* individuals transplanted allopatrically, in this case to colder sites, is likewise observed. The authors argue that the increased fitness can be due to a release from local enemies, such as soil pathogens and below-ground herbivores (De Frenne *et al.*, 2014), which could also be the explanation for our results. As an alternative explanation, *M. effusum* individuals only performed better at ‘Absent’ sites in 2022 after a big mortality event in 2021, which could have acted as a strong selection event. If only the largest, most fit individuals survived and established under the supposedly harsher environmental conditions, these could subsequently outperform, on average, individuals transplanted to more benign sympatric sites where genotypes with lower performance still managed to survive.

Anemone nemorosa *is locally adapted to forest management on a regional scale*

The environmental variables investigated in this study are either tightly linked to and affected by forest management or they are themselves reflecting management intensity (Willems *et al.*, 2021; Møller *et al.*, 2023a). Based on the significant concave relationship of all measured traits with structural complexity in *A. nemorosa*, we have a case of a forest understorey herb clearly showing local adaptation to its sympatric forest patch embedded within a geographic mosaic of environments. Structural complexity is a representation of vertical and horizontal heterogeneity, which is often lacking in forests with high management intensity. A heterogeneous microenvironment can provide selection for phenotypic variation within plant traits and ultimately help conserve genetic diversity (Møller *et al.*, 2023a). In contrast, the lack of a heterogeneous microenvironment in intensively management forests restricts phenotypic plasticity, which becomes evident in our study by the poorer performance of *A. nemorosa* individuals transplanted to high management intensity plots. Furthermore, high management intensity is also associated with more coniferous trees and acidic soils (Møller *et al.*, 2023a). Ultimately, our results indicate that *A. nemorosa* populations are locally adapted to the structural complexity of the site and both species benefit from decreased soil pH. However, linear relationships between measured traits in *A. nemorosa* and SMI imply that understorey herbs potentially rely more on plasticity for phenotypic adjustment along a management gradient.

Land use has been recognized as having one of the strongest impact on species and genetic diversity (IPCC, 2014b). It is critical to test how individuals originating from various management intensities can potentially establish in nonlocal sites for populations to persists in our rapidly changing world. In contrast to previous studies operating at larger scales (De Frenne *et al.*, 2012; Anderson *et al.*, 2013; Toräng *et al.*, 2015), our study takes place on a regional scale, containing a heterogeneous mosaic of forest management intensities and types, and we thus revealed local adaptation at this level as well. The ‘Sympatric & Absent’ comparisons provide useful discernments for the adaptive potential, as they give insights on the dispersal vs. establishment limitations in temperate forests.

Lastly, a recent meta-analysis by Wei et al. (2023) reported that ecological restoration has so far not been successful in recovering the genetic diversity in restored forest ecosystems. Anthropogenic activities such as forest management contributes to the erosion of genetic diversity, and it is critical to preserve the existing genetic diversity proactively, instead of trying to recover it reactively over long periods of restoration time. Genetic diversity is crucial not only for the species own conservation, but the importance also extends to an entire community of dependent organisms (Wimp *et al.*, 2004). This particularly highlights the urgency of promoting forest management practices that aims at maintaining genetic diversity (Møller *et al.*, 2023a; Wei *et al.*, 2023).

Conclusion

In conclusion, forest management and the forest microenvironment confer various selection pressures on understorey herb populations, causing both local adaptation and phenotypic plasticity to help plants to adjust along environmental gradients. The studied forest understorey herbs varied in the amount of phenotypic plasticity and in the ability to establish in foreign sites. Individuals of *A. nemorosa* were found to be locally adapted to the structural complexity of their sympatric site. However, the majority of the measured traits elicited plastic responses when transplanted allopatrically or to foreign sites, indicating that forest understorey herbs generally rely more heavily on phenotypic plasticity for appropriate plant trait response to their environment. Furthermore, our study shows the capacity of forest understorey herbs to establish in nonlocal sites.

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COMPETING INTERESTS

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

CM, PDF and JFS designed the study. CM conducted the field sampling, reciprocal transplant experiment and measurements with the help of MMS. CM analysed the data and wrote the first draft of the manuscript with all authors contributing. Acquisition of the financial support of the project leading to this publication was realized by JFS and PDF.

DATA AVAILABILITY

This work is based on data elaborated by the HerbAdapt and Forest Structure (core) projects of the Biodiversity Exploratories program (DFG Priority Program 1374). The datasets are publicly available in the Biodiversity Exploratories Information System (http://doi.org/10.17616/R32P9Q, https://www.bexis.uni-jena.de/ddm/data/Showdata/22766, and https://www.bexis.uni-jena.de/ddm/data/Showdata/31469).

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Supplementary Material – Manuscript 2

Table S1. *Selected plots from the three regions: Schwäbische Alb (ALB), Hainich-Dün (HAI), and Schorfheide-Chorin (SCH) with their plot numbers within the Biodiversity Exploratories, the corresponding forest management intensity (SMI) as well as the assigned SMI bin. Letters behind bins indicate if the plots were used only for Anemone nemorosa (A), only for Milium effusum (M), or for both (nothing).*

Plot ID	Region	SMI	SMI bin
AEW8	ALB	0.011	Low (A)
AEW12	ALB	0.602	High
AEW15	ALB	0.406	Absent (A)
AEW21	ALB	0.124	Low
AEW25	ALB	0.418	Absent (M)
AEW29	ALB	0.487	High
AEW31	ALB	0.538	Absent (A)
AEW33	ALB	0.528	High
AEW36	ALB	0.421	Mid
AEW37	ALB	0.301	Mid
AEW40	ALB	0.119	Low (M)
AEW41	ALB	0.172	Low
AEW42	ALB	0.241	Mid
AEW44	ALB	0.292	Absent (M)
AEW50	ALB	0.134	Absent
HEW3	HAI	0.508	Absent
HEW9	HAI	0.188	Mid
HEW13	HAI	0.389	Absent
HEW16	HAI	0.333	Mid
HEW17	HAI	0.364	High
HEW19	HAI	0.071	Low
HEW21	HAI	0.135	Low (A)
HEW23	HAI	0.397	High
HEW28	HAI	0.270	Mid (A)
HEW33	HAI	0.047	Low (A)
HEW36	HAI	0.130	Low (M)

HEW44	HAI	0.486	High
SEW6	SCH	0.319	High (A)
SEW7	SCH	0.082	Low (A)
SEW8	SCH	0.059	Low (A)
SEW23	SCH	0.162	Mid (A)
SEW26	SCH	0.051	Absent (A)
SEW35	SCH	0.234	Absent (A)
SEW36	SCH	0.166	Absent (A)
SEW40	SCH	0.250	High (A)
SEW43	SCH	0.229	High (A)
SEW46	SCH	0.088	Mid (A)
SEW47	SCH	0.082	Low (A)
SEW50	SCH	0.116	Mid (A)

Table S2. *Results of a generalized linear mixed-effects model for mortality. Year includes both 2021 and 2022, Transplant category includes ‘Sympatric’, ‘Near allopatric’, ‘Far allopatric’, and ‘Absent’ sites. Species includes Anemone nemorosa and Milium effusum. A binomial error distribution was applied.*

	Df	**Chisq**	**P-value**
Year	1	3.177	0.075
Transplant category	3	4.978	0.173
Species	1	1.613	0.204
Year × Transplant category	3	0.022	0.999
Year × Species	1	0.116	0.734
Transplant category × Species	3	0.274	0.965
Year × Transplant category × Species	3	0.126	0.989

Table S3. Results of mixed-effects models of the measured traits in *A. nemorosa* and *M. effusum* for 2021 at ‘Sympatric & Allopatric’ and ‘Sympatric & Absent’ transplantation sites as response variables, and the linear and quadratic terms of environmental and microclimatic variables as fixed factors. Population, genet, and region were set as random factors. Significant P-values are indicated in bold followed by *P < 0.05; **P < 0.01; ***P < 0.001.

		Anemone nemorosa				*Milium effusum*			
		Sympatric & Allopatric		**Sympatric & Absent**		**Sympatric & Allopatric**		**Sympatric & Absent**	
		χ^2	P-value	χ^2	P-value	χ^2	P-value	χ^2	P-value
Response variable	**Explanatory variable**								
Plant height	SMI	1.341	0.247	1.787	0.181	5.033	**0.025 ***	0.046	0.830
	SMI^2	1.386	0.239	1.247	0.264	0.197	0.657	1.082	0.298
	Structural complexity	1.650	0.199	17.13	**<0.001 *****	6.011	**0.014 ***	1.340	0.247
	Structural complexity^2	0.644	0.422	0.741	0.390	0.076	0.782	1.573	0.210
	Soil pH	0.003	0.960	0.454	0.500	15.02	**<0.001 *****	3.625	0.057
	Soil pH^2	0.106	0.744	1.084	0.298	5.103	**0.024 ***	4.060	**0.044 ***
	Spring temperature	4.070	**0.044 ***	0.022	0.882	7.595	**0.006 ***	0.006	0.937
	Spring temperature^2	0.991	0.319	0.017	0.897	0.002	0.964	1.068	0.301
Number of ramets	SMI	6.648	**0.010 ****	2.751	0.097	4.302	0.038 *	0.126	0.723
	SMI^2	0.427	0.513	1.349	0.245	0.027	0.870	0.001	0.973
	Structural complexity	0.046	0.830	0.058	0.810	1.757	0.185	0.402	0.526
	Structural complexity^2	0.019	0.891	0.439	0.508	2.048	0.152	0.422	0.516
	Soil pH	0.982	0.322	4.470	**0.034 ***	1.209	0.271	1.902	0.168
	Soil pH^2	1.960	0.161	0.101	0.750	0.116	0.733	2.757	0.097
	Spring temperature	0.499	0.480	0.180	0.671	2.001	0.157	0.165	0.684
	Spring temperature^2	0.009	0.924	1.290	0.356	0.019	0.891	0.200	0.655
Performance	SMI	10.11	**0.001 ****	2.148	0.143	3.222	0.073	0.006	0.937
	SMI^2	1.670	0.196	0.837	0.360	0.113	0.736	0.178	0.673
	Structural complexity	1.534	0.215	0.001	0.983	0.279	0.597	1.234	0.267
	Structural complexity^2	0.170	0.680	0.789	0.375	1.926	0.255	0.486	0.486
	Soil pH	1.119	0.290	4.722	**0.030 ***	2.271	0.132	2.172	0.141
	Soil pH^2	0.631	0.427	0.197	0.657	0.085	0.771	2.520	0.112
	Spring temperature	1.692	0.193	0.579	0.447	2.764	0.096	0.389	0.533
	Spring temperature^2	0.149	0.700	0.946	0.331	0.674	0.412	0.427	0.513

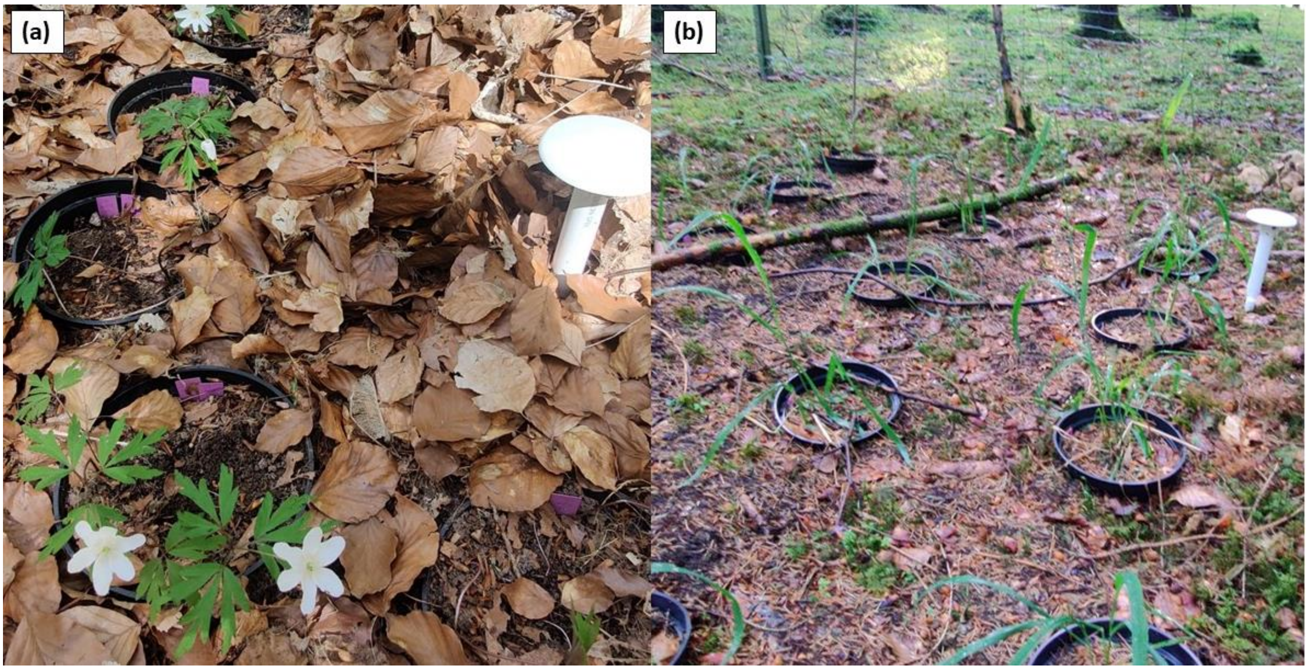

Figure S1. *Photos of the pots in the reciprocal transplant experiment in the field, demonstrating how pots were dug in at ground level of* ***(a)*** *Anemone nemorosa and* ***(b)*** *Milium effusum.*

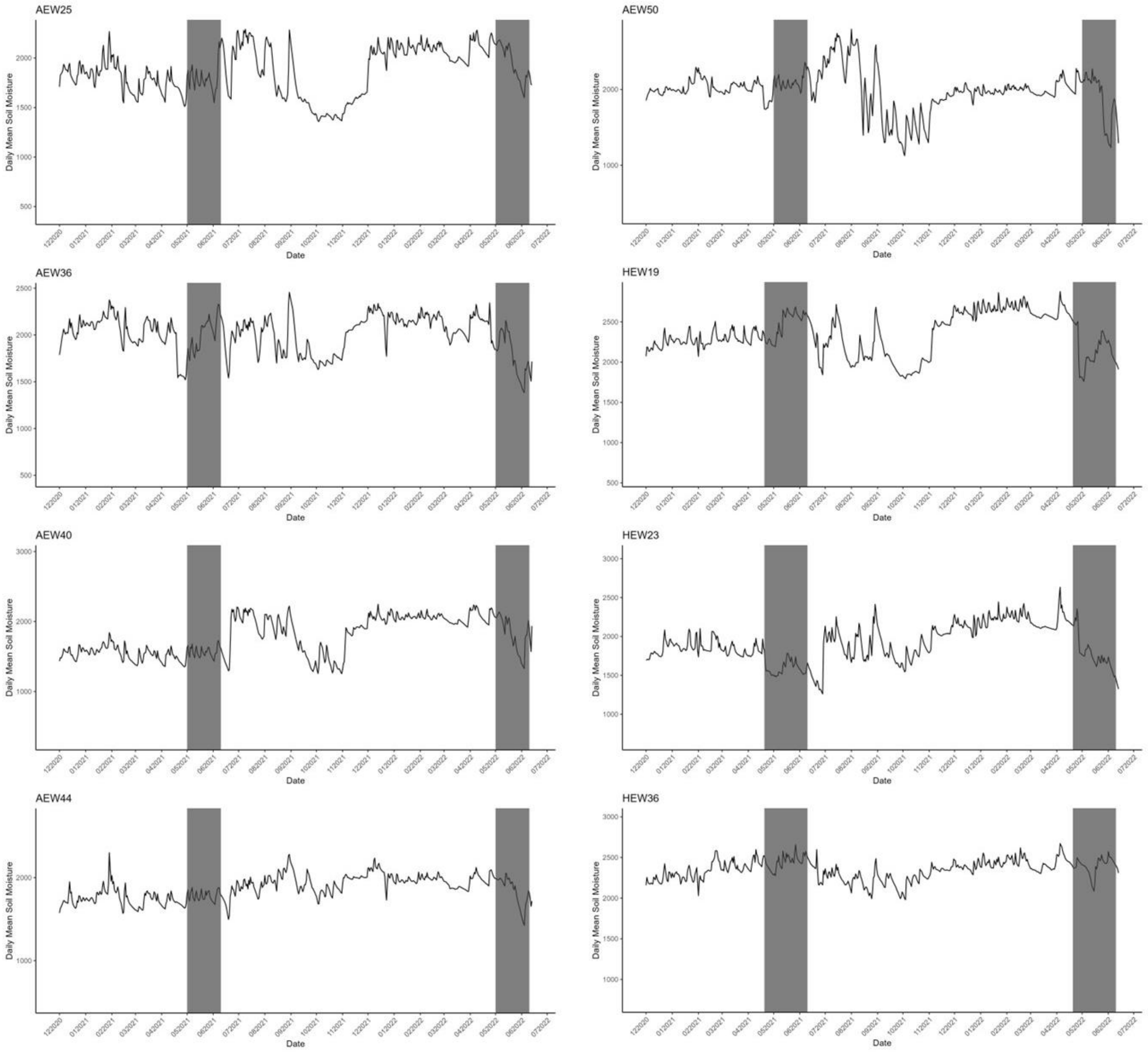

Figure S2. *Soil moisture count from December 2020 to June 2022, at four plots in the Schwäbische Alb (AEW25, AEW36, AEW40, AEW44 and AEW50) and one plot in Hanich-Dün (HEW19, HEW23, and HEW36) where mortality of Milium effusum was high between measurements taken in 2021 and 2022. Data extracted from TOMST TMS4 loggers. Grey highlighted area compare the month of May in 2021 and 2022, indicating a potential drought in 2022.*

CHAPTER 3

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ANNALS OF BOTANY Founded 1887

Intra-individual variation in *Galium odoratum* is affected by experimental drought and shading

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- **Background and aims** Climate-change induced warmer spring temperatures advance tree leaf-out and result in earlier shading of the forest floor. Climate change also leads to more frequent droughts. Forest understorey herbs may respond to these environmental changes by varying traits at different hierarchical levels of organization. While trait mean variation at the inter-individual level in response to environmental changes is well-studied, little is known about how variation at the intra-individual level responds.
- **Methods** We sampled genets of the forest understorey herb *Galium odoratum* from 21 populations in three regions in Germany, varying in microclimatic conditions. The genets were transplanted into a common garden, where we applied shading and drought treatments. We measured plant height and leaf length and width, and calculated the coefficient of variation (CV) at different hierarchical levels: intra-population, intra-genet, intra-ramet and intra-shoot.
- **Key results** Variance partitioning showed that intra-shoot CV represented most of the total variation, followed by intra-ramet CV. We found significant variation in CV of plant height and leaf width among populations of origin, indicating that CV is at least partly genetically based. The soil temperature at populations' origins correlated negatively with CV in plant height, suggesting adaptation to local conditions. Furthermore, we observed that early shade led to increased intra-ramet CV in leaf length, while drought reduced intra-shoot CV in leaf width. Finally, intra-shoot leaf width mean and CV were independent under control conditions but correlated under drought.
- **Conclusions** Our experimental results reveal correlations of intra-individual variation with soil temperature, indicating that intra-individual variation can evolve and may be adaptive. Intra-individual variation responded plastically to drought and shading, suggesting functional changes to improve light capture and reduce evapotranspiration. In conclusion, intra-individual variation makes up the majority of total trait variation in this species and can play a key role in plant adaptation to climatic change.

Key words: Clonal plant, common-garden experiment, forest understorey herbs, *Galium odoratum*, genetic differentiation, intra-individual variation, intra-specific trait variation, microclimate, phenotypic plasticity, sub-individual variation.

INTRODUCTION

Plants consists of repeated homologous structures (e.g. leaves) (Pérez-Harguindeguy *et al.*, 2013), and phenotypic variation in these structures within the same individual is known as intra-individual variation (Herrera, 2009). In clonal plant species, one genet can produce several ramets (i.e. a potentially independent individual derived by vegetative reproduction from a single parent plant) that can vary phenotypically, although arising from the same genotype. Clonal plants are thus perfect for investigating intra-individual variation at several hierarchical levels of biological organization. The intra-individual hierarchical level upwards from the shoot is the ramet, followed by the inter-individual levels: genet and population (Fig. 1). Increasing evidence shows that intra-individual variation may have effects on the overall individual fitness and underlying functional mechanisms (e.g. leaf size may vary within a single individual in response to small-scale variation in the light environment to increase light capture) and may affect population-level variation (Sobral *et al.*, 2013; Herrera *et al.*, 2015; Herrera, 2017; March-Salas *et al.*, 2021). Due to the modular nature of plants, the majority of variation in homologous structures of plants (e.g. fruits, leaves and seeds) may in fact be explained at the intra-individual level (Herrera, 2017). For instance, Herrera *et al.* (2015) found that the intra-shoot level explained ~50 % of both leaf length and leaf width, while the inter-individual level explained <40 % for both traits, and the intra-ramet level explained 5–10 %. Therefore, if intra-individual variation is not considered, large fractions of functional variation and their effects on fitness could potentially be overlooked.

The part of phenotypic variation that is genetically based can be the result of mutation, genetic drift, gene flow or

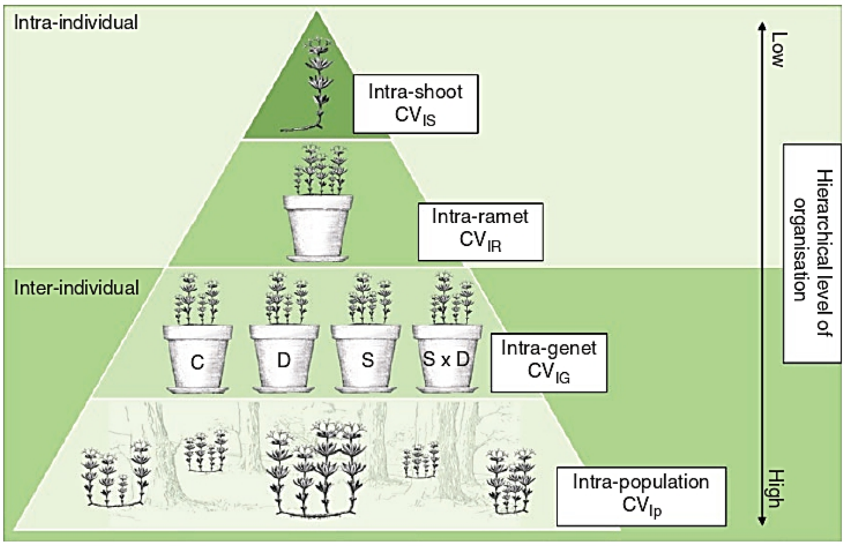

Fig. 1. Schematic figure of the hierarchical levels of organization of *G. odoratum*. In each population, five genets were sampled to calculate intra-population CV. Each genet was separated by cutting its stolons into four separate ramets, each with a single shoot, that were each planted in their own individual pot, which allows calculation of intra-genet CV. Each pot was under one of the four treatments (C, control shade + no drought; S, early shade + no drought; D, control shade + drought; S × D, early shade + drought). Over time, the individually planted ramets in each pot reproduced vegetatively and became a clonally expanding individual made up of several shoots, allowing calculation of intra-ramet and intra-shoot CV. The CV abbreviation is shown at each hierarchical level.

natural selection, with only the latter causing adaptive evolution (Hedrick, 2011). Clonal plants can experience natural selection on traits at several levels – genet, ramet and shoot – rendering measurements of natural selection and predictions of evolutionary responses considerably complex (Fischer and van Kleunen, 2001). Moreover, previous studies have shown that intra-individual variation can be subject to selection within each of these levels (Sobral *et al.*, 2013; Alonso *et al.*, 2018; March-Salas *et al.*, 2021). However, the vast majority of studies on intra-individual variation have been conducted under natural conditions, hampering the assessment of the genetic basis of intra-individual variation. By applying a common garden approach, we can investigate genetic variation in ecologically important traits (Turesson, 1922; De Frenne *et al.*, 2013; Scheepens *et al.*, 2013) as well as genetic variation in the strength of phenotypic plasticity to experimental treatments (West-Eberhard, 2003; Forsman, 2015), although we should be aware that transgenerational non-genetic effects may explain part of this variation (Bossdorf *et al.*, 2008).

While genetic adaptation is important for population survival in the long run, plastic responses are crucial for plant survival of rapid changes in environmental conditions, including those provoked by climate change, although within certain limits. In Concord, North America, tree species have significantly advanced their leaf-out by nearly 2 weeks since the 1850s in response to increasing spring temperatures, whereas forest understorey herbs were much less responsive (Heberling *et al.*, 2019). This phenological mismatch between overstorey trees and understorey herbs is expected to increase with further warming (Chen *et al.*, 2019; Vitasse *et al.*, 2022), suggesting that phenological adjustments may not be sufficient over time and genetic adaptation in trait means and/or plasticity is needed. Additionally, droughts are becoming more frequent with global climate change (Dai, 2013; Büntgen *et al.*, 2021), also in temperate forests (Trenberth *et al.*, 2014; Millar and Stephenson, 2015; Dai *et al.*, 2018), adding yet another stressful condition for understorey plants to overcome. Plants have evolved different ways to cope with drought: they can either escape, avoid or tolerate drought stress (Fang and Xiong, 2015). In response to drought, plants can accelerate their growth and reproductive cycle (escape strategy) (Franks *et al.*, 2007), reduce their mean leaf size and decrease specific leaf area to lower their transpiration rate (avoidance strategy) (Esau, 1977) or make osmotic adjustments and improve water use efficiency (tolerance strategy) (Marron *et al.*, 2003; Liu and Stützel, 2004; Wellstein *et al.*, 2017).

In the above examples, population responses may harbour changes not only in the average trait value of individuals but also in intra-individual variation, for instance in leaf morphology. Larger variation in leaf characteristics and architecture may allow individuals to thrive under variable light and drought conditions. Indeed, capturing different light intensities may help individuals to acclimate to seasonal variation in light conditions, as occurs in the deciduous forest understorey (Martinez and Fridley, 2018). Thus, functional variation within the same individual, such as physiological and morphological variation among leaves, may help individuals and populations to adapt plastically and genetically to fine-grained environmental changes and ultimately favour population performance (Winn, 1996*a*; Sobral *et al.*, 2019). Nevertheless, experiments addressing the causes of intra-individual variation are to our knowledge absent.

Here we examine whether and how inter-individual and intra-individual variation is determined by genetic variation, correlates with microclimatic conditions, and responds to experimental environmental manipulations, i.e. drought and shading treatments. We used the understorey clonal herb *Galium odoratum* as study species to (1) compare variation in three vegetative traits – plant height, leaf length and leaf width – within various hierarchical levels of organization (population, genet, ramet and shoot); (2) test whether inter-individual and intra-individual trait variation has a genetic basis; (3) test if forest management and related micro-environmental factors at the population of origin may have affected genetic variation in individual trait variation; and lastly (4) investigate how experimentally induced drought and earlier shading drive plastic responses in individual trait variation and trait means. By answering these questions, this study provides insightful knowledge on the variation and evolutionary causes of intra-individual variation in the face of future climatic conditions.

MATERIALS AND METHODS

Study species and experimental system

Galium odoratum (L.) Scop. (Rubiaceae) is a perennial forest understorey herb, reaching a height of 10–30 cm. The leaves are lancet-shaped, widest in or just above the middle, and mostly appearing eight at a time, arranged in whorls (Frederiksen and Rasmussen, 2006). Whorls (and the leaves within whorls) typically become bigger with age of the shoot and with increasing distance from the ground. *Galium odoratum* can reproduce sexually via seeds (outcrossing), but also heavily relies on vegetative spread through stolons (Frederiksen and Rasmussen, 2006).

We sampled plants from forest plots (100 m × 100 m) in the Biodiversity Exploratories (www.biodiversity-exploratories.de), a large-scale platform for ecological research in Germany (Fischer *et al.*, 2010). In May 2020, we sampled individuals of *G. odoratum* in three different regions across Germany: Schwäbische Alb (ALB), characterized by calcareous bedrock with an elevation up to 860 m, an annual mean temperature of 6–7 °C and a mean annual precipitation of 700–1000 mm; Hainich-Dün (HAI), also consisting of calcareous bedrock, with a maximum elevation of 550 m, mean annual temperature of 6.5–8 °C and a mean annual precipitation of 500–800 mm; and Schorfheide-Chorin (SCH), which is a young glacial landscape with a maximum elevation of 140 m, annual mean temperature of 8–8.5 °C and mean annual precipitation of 500–600 mm (Fischer *et al.*, 2010). An index for silvicultural management intensity (SMI) has previously been developed to capture forest management intensity (Schall and Ammer, 2013, 2014). We chose nine forest plots (hereafter referred to as populations) along the SMI gradient from low to high management intensity in each region (within regions populations had a distance between each other of 450 m to 28.7 km). Within each region, we sampled five individuals from the local population with a minimum inter-individual distance of 10 m to ensure they were genetically different (hereafter referred to as genets). Each genet was vegetatively propagated, and four ramets were planted into four pots in multitrays (51.5 cm wide, 33.5 cm long, 5.5 cm deep, 54 pots per tray; filled with potting soil CL T torffrei, Einheitserde, Sinntal-Altengronau, Germany) for establishment and growth until November 2020, when all ramets were transferred into 1.5-L pots with potting soil (Typ T, Struktur 1B, Hawita, Vechta, Germany). In spring 2021, all pots were relocated to a foil tunnel allowing us to apply watering and shading treatments. The original design of our experiment included 3 regions × 9 populations × 5 genets × 4 ramets = 540 pots. However, mortality in early spring caused reduced numbers before the start of the experiment, resulting in 135 pots (see Supplementary Data Tables S1 and S2 for final numbers).

The ability of *G. odoratum* to spread vegetatively allowed us to study numerous hierarchical levels of organization: population, genet, ramet and shoot (Fig. 1). A shoot is a potentially independent plant unit and there are usually multiple shoots in a single pot, together making up the ramet. Measurements within both shoots and ramets are considered to reflect variation at the intra-individual level. A genet contains genetically identical ramets, so each of the five individuals that was sampled in each population was a separate genet and these genets were subsequently divided into four different ramets, each planted in a single pot. Since the different ramets in each genet grew in separate pots and experienced different environments (i.e. treatments), the intra-genet level describes inter-individual variation that does not classify as intra-individual level. Populations are the sites of origin where the genets were sampled, and variation within (and among) populations likewise reflects inter-individual variation that does not classify as intra-individual level. All variance on all hierarchical levels studied here is intra-specific variation since it all pertains to trait variation within a single species.

Shading and drought treatments

To simulate the shaded forest understorey environment, we applied shading cloth over the foil tunnel in two layers (45 % shading for each layer, resulting in 90 % total shading). The layers of shading cloth were applied 1 week apart. To simulate future tree leaf-out conditions, the first layer was applied on 12 April 2021, ~2 weeks before the anticipated leaf out of beech and oak trees in the surrounding area. Control shading cloth was applied when the natural leaf-out of surrounding trees was observed in the area (Frankfurt am Main, Germany) on 30 April 2021, likewise applied in two stages, 1 week apart.

We applied the drought treatment at the flowering start of the first plant that flowered (7 May 2021). The drought treatment was applied as a single event, in which all watering was ceased until substantial wilting (50 % of all pots) was observed. This drought treatment lasted for 2 weeks until watering of all pots was resumed. Control plants received water by irrigation from above *ad libitum* during the whole experiment. The shading and drought treatments were applied in a full-factorial design (control, $n = 30$ pots; early shading, $n = 39$; drought, $n = 37$, early shading + drought, $n = 29$).

Measured traits and their variation

We measured plant height, leaf length and leaf width after all plants finished flowering (end of June). Plant height was measured to the nearest 0.5 cm for all shoots in each pot (ranging from 4 to 25 shoots per pot, mean = 17.06 shoots per pot). Leaf length and leaf width were measured with callipers to the nearest 0.1 mm precision on one randomly chosen leaf per whorl, on up to five whorls per shoot (mean number of whorls per shoot = 4.45), on up to five randomly chosen shoots per pot.

The coefficient of variation (CV), calculated as the standard deviation divided by the mean of a specific trait, was used to estimate variation separately in each trait at each hierarchical level of organization: intra-population (CV_{IP}), intra-genet (CV_{IG}), intra-ramet (CV_{IR}) and intra-shoot (CV_{IS}) (Fig. 1). The CV of each level was calculated based on the means and standard deviations of the level beneath (e.g. CV_{IP} was based on the genet trait means and respective standard deviations). Additionally, in order to test whether the CV can be used as an index of intra-class variation (Herrera, 2009), we investigated linear relationships between means and variances for each trait at each hierarchical level (Supplementary Data Fig. S1). The CV_{IS} was not calculated for plant height since one shoot has only a single height value and thus no CV can be calculated.

Data analyses

Firstly, to test which hierarchical level of organization explains the most variation in our measured traits, we performed variance partitioning on the traits using the R package cati and the function partvar (Taudiere and Violle, 2016). Two different variance partitioning models were used. The variance partitioning for plant height only included the nested structure of intra-population, intra-genet, and intra-ramet. The variance partitioning for leaf length and leaf width included all hierarchical levels (intra-population, intra-genet, intra-ramet and intra-shoot). To investigate the robustness of our variance partitioning analysis, we conducted four additional variance partitioning analyses, one for each treatment subset.

Secondly, to investigate the genetic basis of intra-individual variation, we ran linear mixed-effect models (LMMs) for the CV_{IG}, CV_{IR} and CV_{IS} of each trait, using the function lmer from the package lme4 (Bates *et al.*, 2007). We used the hierarchical levels of organization as nested random factors, where the region and population level reflect genetic differentiation among populations, and the genet level is reflecting genetic differences among individuals. For intra-genet level we had no fixed factors and defined the intercept as +1. For intra-ramet and intra-shoot level, shade, drought, and the interaction between the two treatments were included as fixed factors. To test for the genetic basis of intra-individual variation, we performed Akaike information criterion (AIC) model selection using the function AIC on our LMMs. Each level of the nested random factor was removed one at a time, allowing us to compare the AIC of the various models. If the difference in the goodness of fit between two models exceeds an absolute value of 2 (Vrieze, 2012), we interpret the models as being significantly different and the hierarchical level of organization in the model with the lowest AIC value as having importance for explaining CV in the trait and, since the data originate from plants grown under a common garden environment, being at least partly genetically based.

Thirdly, to avoid multicollinearity of environmental variables in the mixed-effects models, we applied a principal component analysis (PCA) from the package factoextra (Kassambara and Mundt, 2017) on the following microclimate variables that were extracted from the BExIS2 database maintained by the Biodiversity Exploratories (Fischer *et al.*, 2010) on the populations of origin: mean annual soil temperature, mean annual air temperature, mean annual humidity and mean annual precipitation. The PCA showed that principal component 1 (PC1) explained 63.8 % of the variation in microclimatic variables, and the primary variable (>36 %) for PC1 was mean annual soil temperature (Supplementary Data Fig. S2).

Fourthly, we tested for treatment effects on CV using LMMs with mean annual soil temperature and the SMI from the origin of each population as fixed effects, including shade, drought and the interaction between the two treatments on the intra-ramet and intra-shoot level. Hierarchical levels of organization were used as a random nested structure (region/population/genet for intra-ramet, and region/population/genet/ramet for intra-shoot). Since the CV at intra-genet level contains all four treatment combinations, we cannot distinguish the treatment effects or analyse this separately on this level. However, intra-genet CV can be regarded as a plasticity index because we have the same genet growing in different conditions. LMMs were run using the function lmer from the package lme4 (Bates *et al.*, 2007) and model test results were obtained by applying the function Anova from the package car (Fox *et al.*, 2012). To investigate significant differences between treatments with more than two levels or significant interactions, a *post hoc* Tukey test was applied using the function lsmeans from the package lsmeans (Lenth, 2016). We also used identical models but with mean trait values of each trait (plant height, leaf length and leaf width) as the response variable (instead of CV).

Lastly, we investigated the relationship between trait means and CV to test whether CV varies independently of trait means. At population and genet level, we ran LMMs with the trait mean as the response variable, CV and quadratic CV of the trait as fixed effects, and nested hierarchical level as random effect. At ramet and shoot level, shade and drought treatments, along with all possible interactions with CV and quadratic CV of the traits were included as fixed effects, with region, population, and genet as nested random effects for ramet level, and the fully nested hierarchical level of organization (i.e. region, population, genet and ramet) as nested random effect for shoot level.

All statistical analyses were conducted with R version 4.1.2 (R Core Team, 2021). Shapiro–Wilk and Bartlett tests were used to assess whether the assumptions of normality and homoscedasticity of model residuals were met, respectively. When at least one of these assumptions was violated, the response variable was transformed. Stepwise backward model selection was applied in all models (Pearce and Ferrier, 2000).

RESULTS

We measured a total of 2304 leaves, from a total of 517 shoots, arising from 135 ramets grown under four different conditions, originating from 71 genets (42 genets provided ramets in at least two treatments), sampled from 21 different populations across the three different regions (ALB, HAI and SCH; Supplementary Data Table S1). Height was measured on all 517 shoots. See Supplementary Data Fig. S3 for trait distributions for each trait on each hierarchical level separately for each treatment.

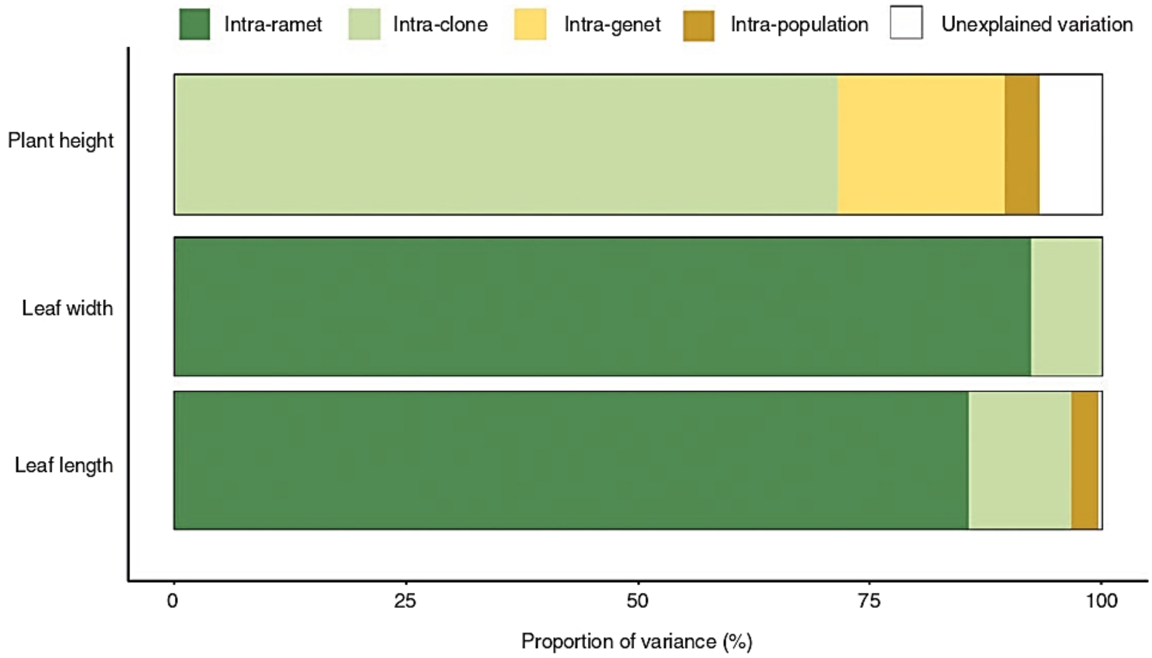

FIG. 2. Proportion of variation in plant height, leaf length and leaf width of *G. odoratum* explained by variance partitioning among different levels of organization: intra-population, intra-genet, intra-ramet, intra-shoot or unexplained.

Variance partitioning on hierarchical levels of organization

Partitions of trait variation into hierarchically nested levels of organization revealed that the variation within shoots (CV_{IS}) was the main source of variation in leaf width and length, accounting for 92.5 and 85.8 % of the total variation, respectively (Fig. 2). For leaf width, 7.5 % was explained by the variation at intra-ramet level, whereas for leaf length the remaining variation, 11.2 %, was mainly explained by the variation at intra-ramet level and 2.8 % by intra-population, and the remaining 0.2 % was unexplained variation (Fig. 2). Variation in height was primarily explained by intra-ramet level with 71.4 %, intra-genet level explained 17.9 %, intra-population explained 3.8 %, and the remaining 6.9 % was unexplained (Fig. 2). For leaf width and leaf length, variance partitioning was robust among treatment subsets and consistent with the overall variance partitioning. When assessing the robustness of the variance partitioning of plant height, the control and early shade treatment subsets showed that intra-population was the main source of variation. For drought and the combined treatment of drought and early shade, intra-ramet and intra-genet mainly explained the majority of the variation (Supplementary Data Fig. S4).

Genetic basis of CV

The hierarchical level of organization with the best goodness of fit, i.e. that best explained CV, varied among traits. At the intra-genet level (reflecting a combination of genetic and plastic effects), AIC values showed that region best explained CV_{IG} in leaf width, and population best explained CV_{IG} in plant height, whereas no differences (≥2) between AIC in models were found for CV_{IG} in leaf length (Table 1). At the intra-ramet level (reflecting genetic effects), none of the hierarchical levels of organization had a significant effect on the CV_{IR} in height, leaf

TABLE 1. *Comparison of models for genetic variation in CV of plant height, leaf length and leaf width in* G. odoratum *at different hierarchical levels. Hierarchical levels were added one at a time as a nested random factor. The full model for intra-genet level had no fixed factors. For intra-ramet and intra-shoot levels, shade, drought and the interaction between the two treatments were included as fixed factors in the full models. Goodness of fit was compared using the AIC. Significantly lower AIC values (ΔAIC ≥ 2) that explain the best goodness of fit are indicated in bold*

Akaike information criterion (AIC) values for genetic basis of trait CV			
	Region	**Region/population**	**Region/population/genet**
Intra-genet (CV_{IG}, $n = 71$)			
Plant height	−123.6	**−126.4**	
Leaf length	−137.8	−136.7	
Leaf width	**−165.3**	−163.3	
Intra-ramet (CV_{IR}, $n = 135$)			
Plant height	−183.6	−183.7	−184.2
Leaf length	−204.1	−205.8	−203.8
Leaf width	−251.8	−250.9	−248.9
Intra-shoot (CV_{IS}, $n = 517$)			
Leaf length	−545.6	−549.8	−549.5
Leaf width	**−609.2**	−607.2	−605.9

length, or leaf width (Table 1). Finally, on the intra-shoot level (reflecting plastic effects), region had the lowest AIC values, and therefore best explained CV_{IS}, in leaf width, whereas population and genet best explained CV_{IS} in leaf length.

Microclimate and treatment effects on CV and mean of measured traits

The CV_{IP}, CV_{IG} and CV_{IR} in height were all significantly affected by mean annual soil temperature (Table 2): CV decreased with increasing mean annual soil temperature (Fig. 3). Additionally, CV_{IP} in leaf length was also significantly negatively affected by mean annual soil temperature. No significant effect of mean annual soil temperature was found on CV in leaf width. No significant effect of SMI was found on CV in any of the vegetative traits.

The early shading treatment significantly increased CV_{IR} in leaf length compared with control shading (Table 2; Fig. 4A). The two-way interaction between shade and drought was significant for CV_{IS} in leaf width (Table 2), with the combination of drought treatment and control shading resulting in lower CV_{IS} compared with the other treatment combinations (Fig. 4B). No significant treatment effects were found in CV in height in any of the hierarchical levels (Table 2). For mean trait values in plant height, leaf length and leaf width, only mean annual soil temperature turned out to be a significant predictor for intra-shoot mean leaf width (Supplementary Data Table S3).

Trait mean and CV relationship

Mean height at intra-genet and intra-ramet level was significantly affected by the quadratic terms of CV_{IG} and CV_{IR} in

TABLE 2. *Results of mixed-effects models of CV in plant height, leaf length and leaf width in* G. odoratum *at different hierarchical levels as the response variables, and the environmental variables mean soil temperature and SMI as fixed factors for all levels, as well as experimental treatments and their interaction as additional fixed factors for intra-ramet and intra-shoot levels. Hierarchical levels of organization were included as random variables in a nested structure. Stepwise backward selection was applied to obtain the most parsimonious models. Parameters used in the minimum adequate model are indicated in bold. If stepwise backward selection did not lead to removal of model parameters, estimates from the full model are shown. Parameter estimates (PE) for continuous variables are indicated with ↑ for positive and ↓ for negative*

Intra-population (CV_{IP}, n = 21)												
	Plant height				**Leaf length**				**Leaf width**			
Predictor	PE	χ^2	d.f.	P	PE	χ^2	d.f.	P	PE	χ^2	d.f.	P
Soil temperature	↓	**5.237**	**1**	**0.022***	↓	**3.870**	**1**	**0.049***	↓	2.844	1	0.092
SMI	↑	0.546	1	0.460	↓	0.036	1	0.850	↑	0.054	1	0.817
Intra-genet (CV_{IG}, n = 71)												
	Plant height				**Leaf length**				**Leaf width**			
Predictor	PE	χ^2	d.f.	P	PE	X^2	d.f.	P	PE	χ^2	d.f.	P
Soil temperature	↓	**7.682**	**1**	**0.006****	↓	1.012	1	0.314	↓	0.130	1	0.718
SMI	↓	0.045	1	0.831	↑	0.482	1	0.488	↑	1.616	1	0.204
Intra-ramet (CV_{IR}, n = 135)												
	Plant height				**Leaf length**				**Leaf width**			
Predictor	PE	χ^2	d.f.	P	PE	χ^2	d.f.	P	PE	χ^2	d.f.	P
Soil temperature	↓	**8.686**	**1**	**0.003****	↓	0.658	1	0.417	↓	0.050	1	0.823
SMI	↓	0.077	1	0.782	↑	0.002	1	0.965	↑	0.477	1	0.490
Shade (S)		2.250	1	0.134		**4.299**	**1**	**0.038***		3.127	1	0.077
Drought (D)		0.046	1	0.831		0.662	1	0.416		1.407	1	0.236
S × D		1.813	2	0.404		0.004	1	0.948		0.510	1	0.475
Intra-shoot (CV_{IS}, n = 517)												
					Leaf length				**Leaf width**			
Predictor					PE	χ^2	d.f.	P	PE	χ^2	d.f.	P
Soil temperature					↓	0.799	1	0.371	↓	0.869	1	0.351
SMI					↓	0.308	1	0.579	↓	0.031	1	0.861
Shade (S)						2.592	1	0.107		**2.348**	**1**	**0.125**
Drought (D)						1.254	1	0.263		**1.508**	**1**	**0.220**
S × D						*3.219*	*1*	*0.073*		**6.229**	**1**	**0.012***

Significance levels are represented by asterisks: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

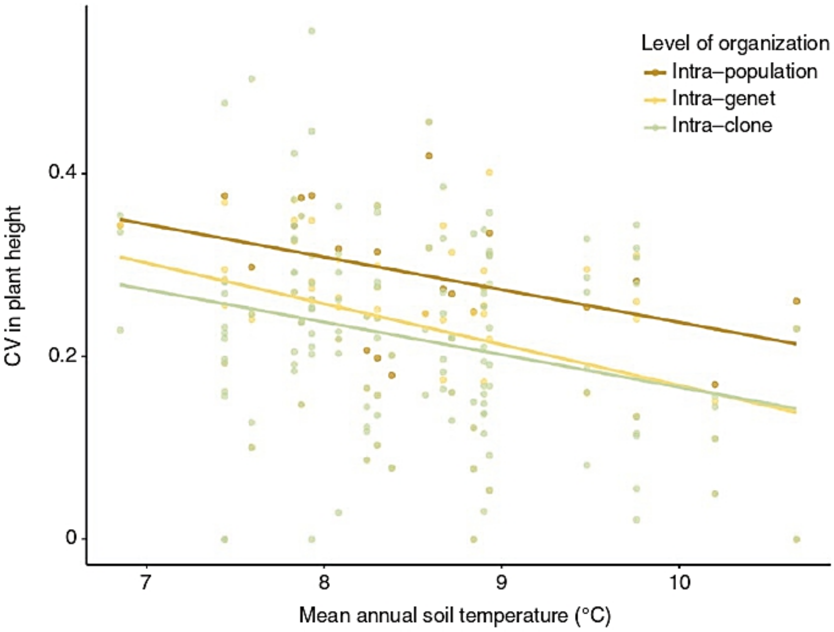

FIG. 3 Relationship between mean annual soil temperature at the population of origin with the intra-individual variation in height in *G. odoratum*. Regression lines through data (dots) from different hierarchical levels of organization are shown.

height (Table 3). Mean leaf length was significantly affected by the quadratic terms of CV_{IG}, CV_{IR} and CV_{IS}. Mean leaf width was significantly affected by the CV_{IG}, CV_{IR} and CV_{IS} of leaf width. Furthermore, our results show a significant interaction for mean leaf length on the intra-ramet level between quadratic CV_{IR} and drought treatment (Table 3). Specifically, mean leaf length and quadratic CV_{IR} of leaf length were related under the drought treatment, but they were independent under the control treatment (Fig. 5).

DISCUSSION

This experimental study using *G. odoratum* as model species revealed that (1) intra-shoot variation, which is the lowest hierarchical level in our system, explains the vast majority of overall leaf trait variation in the populations, followed by intra-ramet variation – these two levels represent intra-individual variation, and our results thus confirm that intra-individual variation can exceed inter-individual variation, as previously observed (Herrera, 2017); (2) inter-individual and intra-individual trait variation at different scales is partly genetically based; (3) this variation may have been the result of selection by microclimatic conditions in the populations of origin; (4) intra-shoot and intra-ramet variations in leaf traits vary under induced drought and early shading; and (5) drought also led to a dependent relationship between mean leaf size and intra-individual variation in leaf size.

Variance partitioning across hierarchical levels of biological organization

Our variance partitioning analysis showed that the lowest hierarchical level of organization, intra-shoot variation, explained the majority of total variation in leaf length and leaf width, followed by intra-ramet variation. Additionally, variation in plant height was primarily explained by the intra-ramet level, again the lowest level of organization analysed. Although the differences in the variance partitioning analyses for the four treatment subsets for plant height suggest weak robustness, they also suggest that the environmental conditions could influence which hierarchical level has most influence on CV in plant height, and it seems that when a drought is imposed, low hierarchical levels are responding more strongly in terms of CV. Overall, this is congruent with what has been observed in previous studies, where intra-shoot (or intra-individual) variation also explained more variation in leaf traits, fruits and seeds than the variation among plants (Herrera *et al.*, 2015; Herrera, 2017). These patterns in the two lowest hierarchical levels could be due to leaf trait changes through time while the plant develops, whereas at higher hierarchical levels the trait differences are already summarized across shoots and therefore average out the intra-shoot variation (i.e. leaves on different shoots are on average more similar to each other than leaves within a shoot). However, due to the nature of the experimental design, effects of the treatments applied on the intra-genet level can potentially affect the intra-genet variation. Even though the treatments increased the variation at the intra-genet level, the proportions of explained variance are still higher on our two lowest hierarchical levels: intra-ramet and intra-shoot. Taken together, these results support the notion that intra-individual variation is responsible for the larger share of the total variation, potentially driving population performance and plastic responses to environmental changes.

Genetic differentiation in intra-individual variation

In our common garden experiment, any differences in intra-ramet and intra-shoot CV among regions, populations or

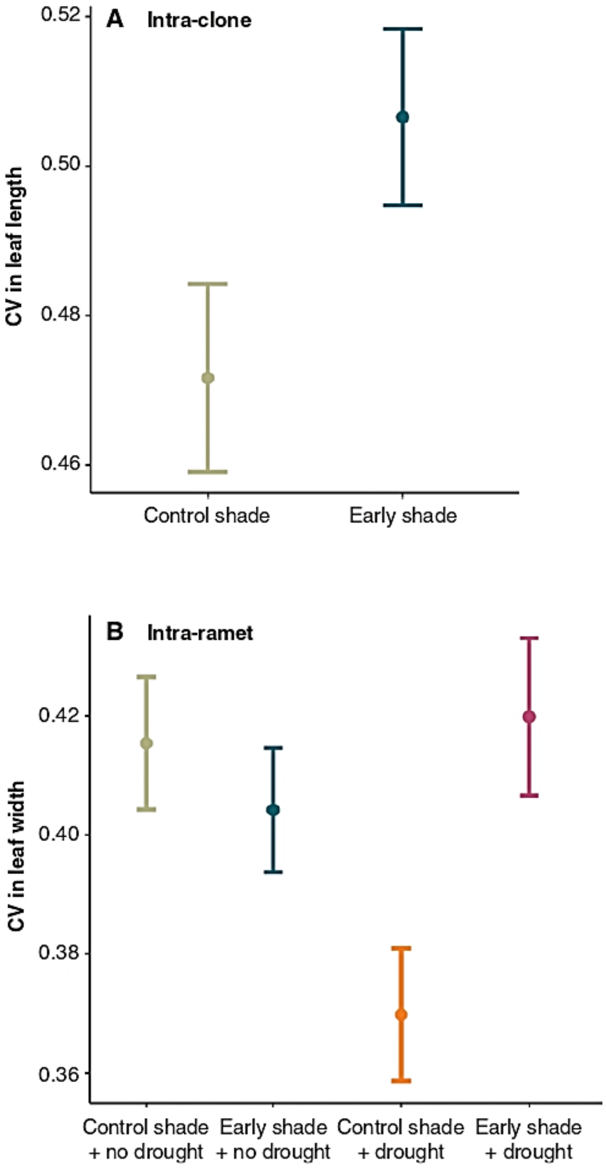

FIG. 4. Shading and drought treatment effects on the CV at two intra-individual levels in *G. odoratum*. (A) Effect of control and early shade treatment on CV in leaf length at the intra-ramet level. (B) Effect of the four different treatment combinations on CV in leaf width at intra-shoot level.

genets of origin suggest the existence of genetically, epigenetically or perhaps otherwise transgenerational plasticity-based phenotypic variation in intra-individual variation (Herrera *et al*., 2022; Sobral and Sampedro 2022). We revealed a general pattern of the highest hierarchical levels of region and population having the best goodness of fit, thereby being the primary factors explaining genetic variation in CV at the hierarchical levels of intra-genet and intra-shoot. This pattern could well reflect the strong versatility of clonal plants, allowing individuals to adjust their life-history in terms of vegetative reproduction, the placement of shoots and the degree of integration between shoots according to the local environmental conditions (Fischer and van Kleunen, 2001). An individual from a fine-grained heterogeneous environment and microclimate is more likely to invest in increased variation in order to deal with environmental changes and predictability on a small spatial scale, compared with an individual from a coarse-grained heterogeneous or even homogenous environment (Winn, 1996*b*; Stark *et al*., 2017). Despite the lack of any Significant improvement in goodness of fit at the intra-ramet level, the observed patterns are consistent with both intra-genet and intra-shoot level. The observed variation in the CV of plant height, leaf length and width, primarily explained by region and population, may very well have been driven by the different environments among regions (ALB, HAI and SCH) and among all sampled populations (see next section).

Effects of microclimate of origin and treatments on CV

Mean annual soil temperature from the populations of origin was found to have a significant effect on variation in height at all hierarchical levels of organization, as well as on variation in leaf length at intra-population level. The negative association between CV in height and mean annual soil temperature indicates that individuals originating from colder sites show increased variation; this supports a stress-induced variability hypothesis stating that abiotic stress increases intra-specific variation (Kuppler *et al*., 2020) and is coherent with previous studies (Sobral *et al*., 2019). Colder plots from the Biodiversity Exploratories are primarily within coniferous forests, resulting in more or less constant shaded conditions (Willems *et al*., 2021). Therefore, a possible explanation for the observed increased height variation in individuals originating from colder plots could be that these individuals have evolved an optimized way to capture light under light-limited conditions by increasing their variation, potentially by number of leaves and the positioning of them (Valladares and Brites, 2004). This relationship thus suggests that intra-individual variation may respond adaptively to various environmental conditions (Herrera *et al*., 2015) and may ultimately lead to adaptation to long-term microclimatic conditions (May *et al*., 2017).

At the intra-ramet level, variation in leaf length was found to increase under 2-weeks-earlier shading, indicating a rapid phenotypic change within clonal plants in response to environmental drivers. Under shaded conditions, common phenotypic plastic responses are an increased mean leaf size and specific leaf area (Pigliucci and Kolodynska, 2002; Sultan, 2003). After shading was applied, leaf length values may have adjusted in emerging whorls on the developing shoots, causing the intra-individual variation to increase under the early shading treatment. However, the intra-shoot variation in leaf width of individuals exposed to the control shading with drought was considerably lower compared with the rest of the treatments. Possibly, the increase in intra-individual variation observed in the early shading with drought treatment could be a result of plant responses in various directions in response to drastic environmental conditions (Winn, 1996*b*; Sun *et al*., 2021), potentially benefitting the individual's performance under the drought conditions, perhaps through facilitating drought tolerance. Another strategy to deal with drought is to

TABLE 3. *Results of mixed-effects models of trait means in plant height, leaf length and leaf width in* G. odoratum *at different hierarchical levels as the response variables, and the corresponding CV and CV^2 as fixed factors. Shade, drought and all possible interactions were additionally included as fixed factors on the intra-ramet and intra-shoot levels. Hierarchical levels of organization were included as random variables in a nested structure. Stepwise backward selection was applied to obtain the most parsimonious models. Parameters used in the final model are indicated in bold. If the stepwise backward selection did not lead to removal of parameters in the model, estimates from the full model are shown. No significant results were found for intra-population CV (CV_{IP}), and thus this level is not shown*

Intra-genet (n = 71)									
	Plant height			**Leaf length**			**Leaf width**		
Predictor	χ^2	d.f.	***P***	χ^2	d.f.	***P***	χ^2	d.f.	***P***
Trait CV_{IG}	3.453	1	0.063	0.604	1	0.437	**7.395**	**1**	**0.007****
Trait CV_{IG}^2	**4.178**	**1**	**0.041***	**6.386**	**1**	**0.012***	0.023	1	0.880
Intra-ramet (n = 135)									
	Plant height			**Leaf length**			**Leaf width**		
Predictor	χ^2	d.f.	*P*	χ^2	d.f.	*P*	χ^2	d.f.	*P*
Trait CV_{IR}	2.482	1	0.115	1.173	1	0.279	**20.149**	**1**	**<0.001*****
Trait CV_{IR}^2	**3.951**	**1**	**0.047***	**10.965**	**1**	**<0.001*****	0.006	1	0.937
Shade (S)	0.430	1	0.512	0.790	1	0.374	0.359	1	0.549
Drought (D)	0.002	1	0.962	**1.656**	**1**	**0.198**	1.137	1	0.286
Trait CV_{IR} × S	0.258	1	0.612	0.010	1	0.929	0.098	1	0.754
Trait CV_{IR} × D	0.363	1	0.547	0.121	1	0.728	0.080	1	0.777
Trait CV_{IR}^2 × S	0.386	1	0.534	0.551	1	0.458	0.174	2	0.917
Trait CV_{IR}^2 × D	0.081	1	0.776	**4.705**	**1**	**0.030***	0.224	2	0.894
Trait CV_{IR} × S × D	0.338	1	0.561	0.068	1	0.794	0.515	2	0.773
Trait CV_{IR}^2 × S × D	1.937	3	0.586	2.611	2	0.271	1.053	4	0.902
Intra-shoot (n = 517)									
				Leaf length			**Leaf width**		
Predictor				χ^2	d.f.	*P*	χ^2	d.f.	*P*
Trait CV_{IS}				0.087	1	0.768	**112.22**	**1**	**< 0.001*****
Trait CV_{IS}^2				**67.162**	**1**	**< 0.001*****	0.760	1	0.384
Shade (S)				0.434	1	0.510	0.646	1	0.422
Drought (D)				3.071	1	0.080	1.995	1	0.158
Trait CV_{IS} × S				0.002	1	0.965	0.001	1	0.970
Trait CV_{IS} × D				0.521	1	0.771	0.014	1	0.907
Trait CV_{IS}^2 × S				0.215	1	0.643	0.331	1	0.565
Trait CV_{IS}^2 × D				0.004	1	0.950	1.524	2	0.469
Trait CV_{IS} × S × D				0.036	2	0.982	0.050	2	0.975
Trait CV_{IS}^2 × S × D				0.533	2	0.766	0.050	1	0.823

Significance levels are represented by asterisks: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

avoid unnecessary evapotranspiration by minimizing leaf area (Marron *et al.*, 2003; Liu and Stützel, 2004; Wellstein *et al.*, 2017). Newly developing leaves have the opportunity to respond to current environmental conditions, given that there is sufficient phenotypic plasticity for rapid adjustments. However, the low intra-shoot variation in leaf width observed under the drought treatment implies that the individuals did not respond to the drought conditions by further minimizing their leaf area, leading to the low intra-individual variation.

An important limitation of this study is that we cannot disentangle treatment effects on the higher hierarchical levels of organization. The treatments were applied at the intra-genet level, one treatment per ramet, and these ramets were subsequently used to calculate the CV for the genet. However, this also means that whatever CV was observed at the intra-genet level was likely to be largely due to phenotypic plasticity.

With regard to intra-individual responses, it is important to consider the morphology of the study species. *Galium odoratum* has its leaves positioned in multiple whorls on a single shoot. These whorls and leaves generally increase in size with increasing distance from the ground surface, with the exception of the top whorl, which is typically still under development. Therefore, some variation would always be present, even under a hypothetically constant environment, simply

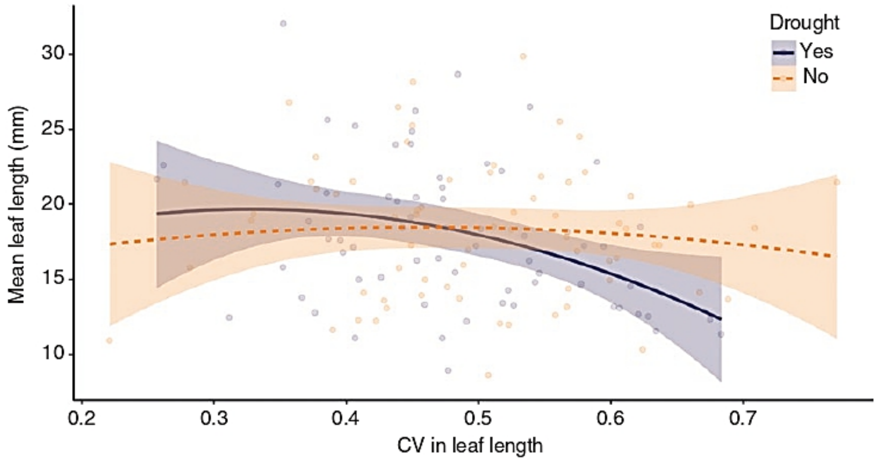

Fig. 5. Significant two-way interaction between drought treatment and quadratic CV in leaf length on mean leaf length in *G. odoratum* at intra-ramet level. The dashed orange curve represents the control treatment while the blue curve represents the drought treatment; 95 % confidence intervals are shown around the curves.

due to the structure and architecture of the leaves and whorls (Fischer and van Kleunen, 2001). Nevertheless, this inherent intra-individual variation could very well be a result of evolution of *G. odoratum*, for instance to maximize light capture (Chazdon and Kaufmann, 1993; Reich *et al.*, 2003).

Relationship between trait means and CV

Significant relationships between trait means and CV would suggest that these two components are interdependent and that one could drive the other. However, if means and CV are independent, intra-individual variation should be considered as an individual property in itself (Herrera, 2017), able to respond and evolve unconstrained with respect to mean trait values. We observed both linear and quadratic relationships between trait means and the CV on intra-genet, intra-ramet and intra-shoot level, but also in some cases an absence of any relationship. The quadratic functions suggest a more complex relationship, where substantially low and high intra-individual variation values reduce mean plant responses while intermediate intra-individual variation values could enhance a plant's performance, in line with stabilizing selective regimes found in previous studies (March-Salas *et al.*, 2021). In this sense, the optimal strategy for some plants could be to invest in intermediate intra-individual variation.

At the intra-ramet level, we found that the relationship between mean leaf length and leaf length CV depends on the drought treatment: under the control conditions there were no relationships between mean leaf length and its variation, whereas under drought conditions there existed a slight concave relationship that showed decreasing mean leaf length with increasing intra-individual variation. Thus, under drought stress, plants with decreasing leaf length become less uniform in their leaf traits. This difference between treatment responses indicates that drought conditions affect the relationship between trait means and their variation. In this sense, it could be that plants promote intra-individual changes to optimize final plant trait values, or potentially facilitate eco-physiological traits through water use efficiency and decreased evapotranspiration under stressful conditions (Marron *et al.*, 2003; Liu and Stützel, 2004; Wellstein *et al.*, 2017). Smaller plants may therefore be particularly prone to increase their intra-individual variation in order to adjust to the stressful drought conditions due to their smaller leaf sizes.

Conclusions

In sum, using a common garden experiment we demonstrated that intra-individual variation introduces an important component of variation in populations. Intra-shoot variation explained the vast majority of total population variation, suggesting a relevant role of phenotypic variation within individuals for adjustments to the local environmental conditions and ultimately for population performance. Furthermore, our results indicate that intra-individual variation in vegetative traits may be partially genetically based, indicating that it could be under the influences of neutral or selective evolutionary processes. Relationships between mean annual soil temperature of the populations' origin and intra-individual variation in vegetative traits suggest that the environment shaped potentially genetically based among-population variation in intra-individual variation. We also showed that intra-individual variation responds plastically to drought and shading treatments, which may allow plants to maintain or improve overall performance under stressful or varying environmental conditions. However, we lack strong support for mechanistic explanations for most patterns in intra-individual variation, potentially mediated by eco-physiological and epigenetic modifications. We therefore conclude that, overall, the observed patterns in intra-individual variation suggest its important role in genetic adaptation to microclimatic variation among populations and in plastic adjustments to rapidly changing microclimatic conditions within populations.

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. Table S1: overview of the distribution of the clonal individuals included in the final study design, the treatments they were subjected to, the number of genets they originated from and the number of populations that had successfully been sampled in each of the three regions. Table S2: overview of the final distribution of the populations of origin, the surviving genets collected there and the treatment combinations that were covered in the final statistical analyses. Table S3: results of mixed-effects models of trait means in height, leaf length and leaf width in *G. odoratum* at different hierarchical levels as the response variables, and the environmental variables mean soil temperature and silvicultural management intensity as fixed factors for all levels, as well as experimental treatments and their interaction as the explanatory variables for intra-ramet and intra-shoot. Figure S1: linear relationships between trait means and CVs for leaf width, leaf length and plant height for each hierarchical level of organization. Figure S2: visualization of PC1 and PC2 axes from the PCA on the microclimatic variables of the 21 sampled populations and contribution of each microclimatic variable to the PC1 axis. Figure S3: histograms for CV in plant height, leaf length and leaf width on each hierarchical level of organization for each treatment combination. Figure S4: proportion of variation in leaf length, leaf width and plant height in *G. odoratum* explained by variance partitioning among different levels of organization: intra-population, intra-genet, intra-ramet, intra-shoot or unexplained variation, for each treatment combination.

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Supplementary Material – Publication 1

Table S1. *Overview of the distribution of the clonal individuals included in the final study design, the treatments they were subjected to, number of genets they originated from and number of populations that had successfully been sampled in each of the three regions.*

	Populations (N)	Genets (N)	Treatments	Ramets (N)
Schwäbian Alb (ALB)	8	26	Control	12
			Early Shading	10
			Drought	14
			Early Shading + Drought	7
Hainich-Dün (HAI)	8	27	Control	12
			Early Shading	15
			Drought	14
			Early Shading + Drought	17
Schorfheide-Chorin (SCH)	5	18	Control	6
			Early Shading	14
			Drought	9
			Early Shading + Drought	5
Total	**21**	**71**	Control	30
			Early Shading	39
			Drought	37
			Early Shading + Drought	29
				135

Table S2. *Overview of the final distribution of the populations of origin, the surviving genets collected there and the treatment combinations that were covered in the final statistical analyses.*

Region	Population ID	Genet ID	Treatment
ALB	A12	3	Drought
ALB	A12	2	Early shade + Drought
ALB	A21	2	Early shade + Drought
ALB	A21	4	Drought
ALB	A21	4	Control
ALB	A21	3	Early shade
ALB	A21	5	Early shade
ALB	A29	3	Early shade
ALB	A29	4	Drought
ALB	A29	3	Control
ALB	A29	2	Control
ALB	A33	5	Early shade + Drought
ALB	A33	3	Early shade
ALB	A33	1	Early shade
ALB	A33	1	Early shade + Drought
ALB	A33	2	Drought
ALB	A33	3	Drought
ALB	A33	2	Control
ALB	A33	5	Control
ALB	A33	1	Control
ALB	A33	2	Early Shade
ALB	A33	3	Early shade + Drought
ALB	A36	1	Early shade
ALB	A36	2	Drought
ALB	A37	3	Early shade + Drought
ALB	A37	3	Drought
ALB	A37	3	Control
ALB	A37	2	Control
ALB	A37	1	Control
ALB	A37	3	Early shade
ALB	A41	5	Drought
ALB	A41	2	Control
ALB	A41	4	Drought

ALB	A41	2	Early shade
ALB	A42	1	Early shade + Drought
ALB	A42	3	Early shade
ALB	A42	4	Drought
ALB	A42	2	Drought
ALB	A42	1	Control
ALB	A42	1	Drought
ALB	A42	3	Drought
ALB	A42	5	Drought
ALB	A42	2	Control
HAI	H13	5	Drought
HAI	H13	5	Control
HAI	H13	5	Early shade + Drought
HAI	H16	2	Early shade
HAI	H16	5	Early shade + Drought
HAI	H16	1	Drought
HAI	H16	5	Control
HAI	H16	4	Drought
HAI	H16	3	Control
HAI	H16	3	Early shade
HAI	H16	5	Early shade
HAI	H16	1	Early shade + Drought
HAI	H16	1	Early shade
HAI	H19	3	Early shade + Drought
HAI	H19	3	Early shade
HAI	H21	5	Early shade + Drought
HAI	H21	4	Early shade + Drought
HAI	H21	1	Drought
HAI	H21	5	Control
HAI	H21	4	Drought
HAI	H21	5	Early shade
HAI	H21	1	Early shade + Drought
HAI	H23	4	Early shade + Drought
HAI	H23	5	Control
HAI	H23	1	Drought
HAI	H23	3	Drought
HAI	H28	1	Early shade + Drought
HAI	H28	4	Early shade + Drought

HAI	H28	2	Early shade
HAI	H28	4	Drought
HAI	H28	1	Control
HAI	H28	3	Drought
HAI	H28	2	Control
HAI	H28	4	Control
HAI	H28	3	Early shade
HAI	H28	4	Early shade
HAI	H28	1	Early shade
HAI	H33	3	Early shade + Drought
HAI	H33	4	Early shade
HAI	H33	2	Early shade
HAI	H33	2	Early shade + Drought
HAI	H33	3	Control
HAI	H33	2	Drought
HAI	H33	4	Drought
HAI	H33	1	Early shade + Drought
HAI	H44	3	Early shade
HAI	H44	2	Early shade + Drought
HAI	H44	4	Early shade + Drought
HAI	H44	1	Drought
HAI	H44	4	Drought
HAI	H44	1	Control
HAI	H44	4	Control
HAI	H44	3	Drought
HAI	H44	2	Control
HAI	H44	2	Early shade
HAI	H44	4	Early shade
HAI	H44	1	Early shade + Drought
HAI	H44	5	Early shade + Drought
SCH	S24	4	Early shade
SCH	S24	1	Early shade
SCH	S24	3	Early shade + Drought
SCH	S24	4	Drought
SCH	S24	3	Control
SCH	S24	5	Control
SCH	S24	2	Control
SCH	S24	2	Early shade

SCH	S40	1	Drought
SCH	S40	5	Early shade
SCH	S40	4	Early shade + Drought
SCH	S40	1	Early shade
SCH	S47	2	Early shade + Drought
SCH	S47	4	Early shade
SCH	S47	5	Early shade
SCH	S47	5	Drought
SCH	S47	1	Drought
SCH	S47	3	Control
SCH	S47	3	Drought
SCH	S47	5	Control
SCH	S47	4	Control
SCH	S47	3	Early shade
SCH	S47	1	Early shade + Drought
SCH	S47	2	Early shade
SCH	S47	1	Early shade
SCH	S49	5	Drought
SCH	S49	2	Early shade
SCH	S7	2	Early shade + Drought
SCH	S7	2	Early shade
SCH	S7	2	Drought
SCH	S7	5	Drought
SCH	S7	5	Early shade
SCH	S7	1	Early shade

Table S3. *Results of mixed-effects models of trait means in height, leaf length and leaf width in Galium odoratum at different hierarchical levels as the response variables, and the environmental variables mean soil temperature and silvicultural management intensity (SMI) as fixed factors for all levels, as well as experimental treatments and their interaction as the explanatory variables for intra-ramet and intra-shoot. Hierarchical levels of organisation were used as random nested structure. Stepwise backwards selection was applied to obtain the most parsimonious models. Parameters used in the minimum adequate model are indicated with bold. If stepwise backwards selection did not lead to removing model parameters, estimates from the full model are shown. Parameter estimates (PE) for continuous variables are indicated with ↑ for positive and ↓ for negative. Chi-square (X^2) test values, degrees of freedom (df) and P-values (P) are given.*

INTRA-POPULATION (N = 21)												
	Mean height				**Mean leaf length**				**Mean leaf width**			
Predictors	PE	X^2	df	P	PE	X^2	df	P	PE	X^2	df	P
Soil temperature	↑	2.617	1	0.106	↑	1.111	1	0.292	↑	3.464	1	0.063
SMI	↑	1.188	1	0.276	↑	0.247	1	0.619	↑	1.215	1	0.270
INTRA-GENET (N = 71)												
	Mean height				**Mean leaf length**				**Mean leaf width**			
Predictors	PE	X^2	df	P	PE	X^2	df	P	PE	X^2	df	P
Soil temperature	↑	1.219	1	0.270	↓	0.000	1	0.988	↑	2.205	1	0.138
SMI	↑	0.092	1	0.762	↑	0.119	1	0.730	↑	0.490	1	0.484
INTRA-RAMET (N = 135)												
	Mean height				**Mean leaf length**				**Mean leaf width**			
Predictors	PE	X^2	df	P	PE	X^2	df	P	PE	X^2	df	P
Soil temperature	↑	0.727	1	0.394	↑	0.007	1	0.932	↑	2.718	1	0.099
SMI	↓	0.008	1	0.928	↓	0.016	1	0.901	↑	0.150	1	0.699
Shade (S)		0.913	1	0.339		1.515	1	0.219		1.621	1	0.203
Drought (D)		0.029	1	0.866		1.101	1	0.294		0.635	1	0.425
S × D		0.491	1	0.484		1.363	1	0.243		0.462	1	0.497
INTRA-SHOOT (N = 517)												
					Mean leaf length				**Mean leaf width**			
Predictors					PE	X^2	df	P	PE	X^2	df	P
Soil temperature					↑	0.175	1	0.676	↑	**4.605**	**1**	**0.032 ***
SMI					↑	0.001	1	0.973	↑	0.124	1	0.725
Shade (S)						1.526	1	0.213		0.995	1	0.318
Drought (D)						1.173	1	0.279		1.074	1	0.300
S × D						0.642	1	0.423		0.265	1	0.607

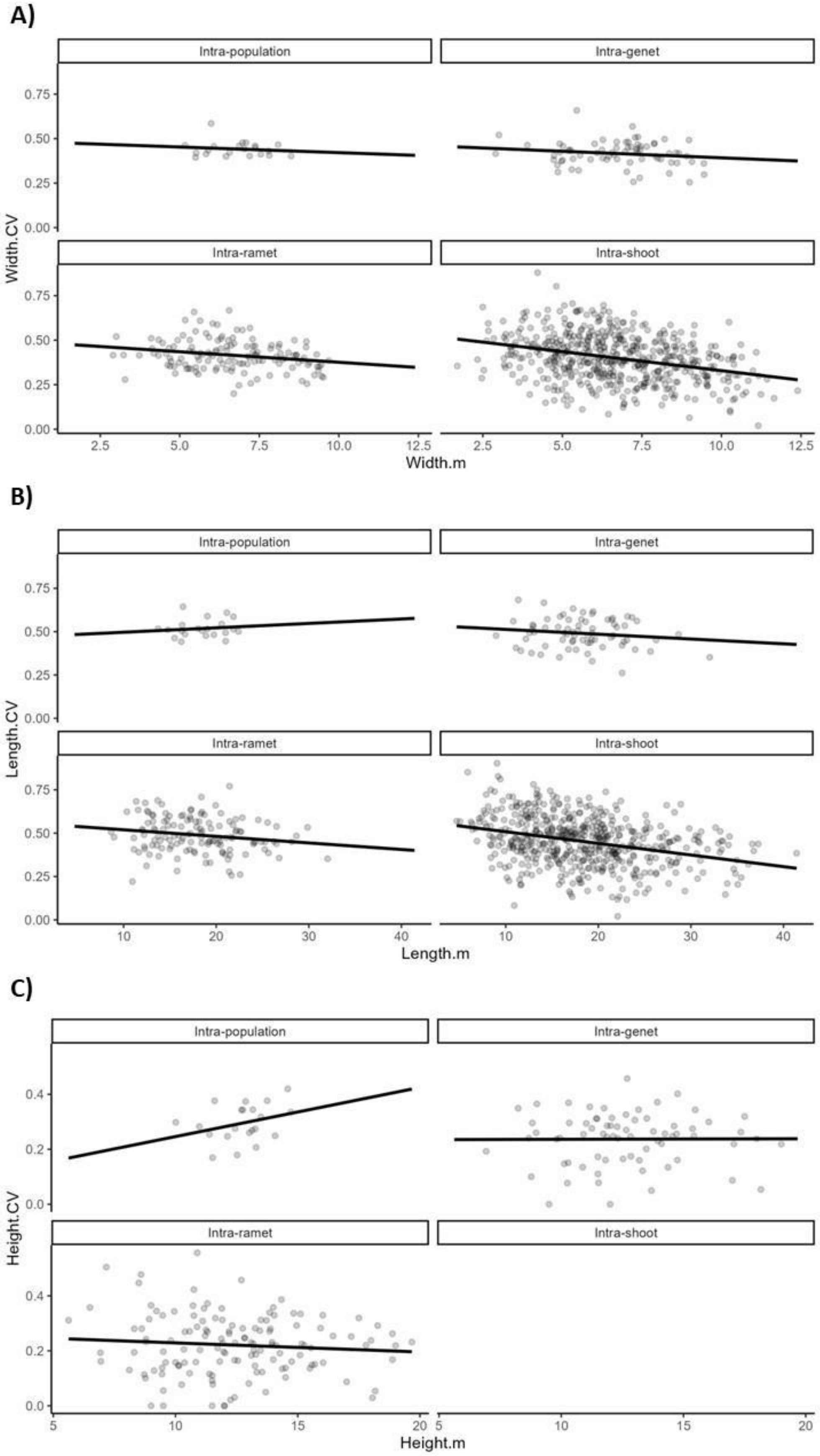

Figure S3: *Linear relationships between trait means and coefficient of variations for A) leaf width; B) leaf length; and C) plant height, for each hierarchical level of organisation.*

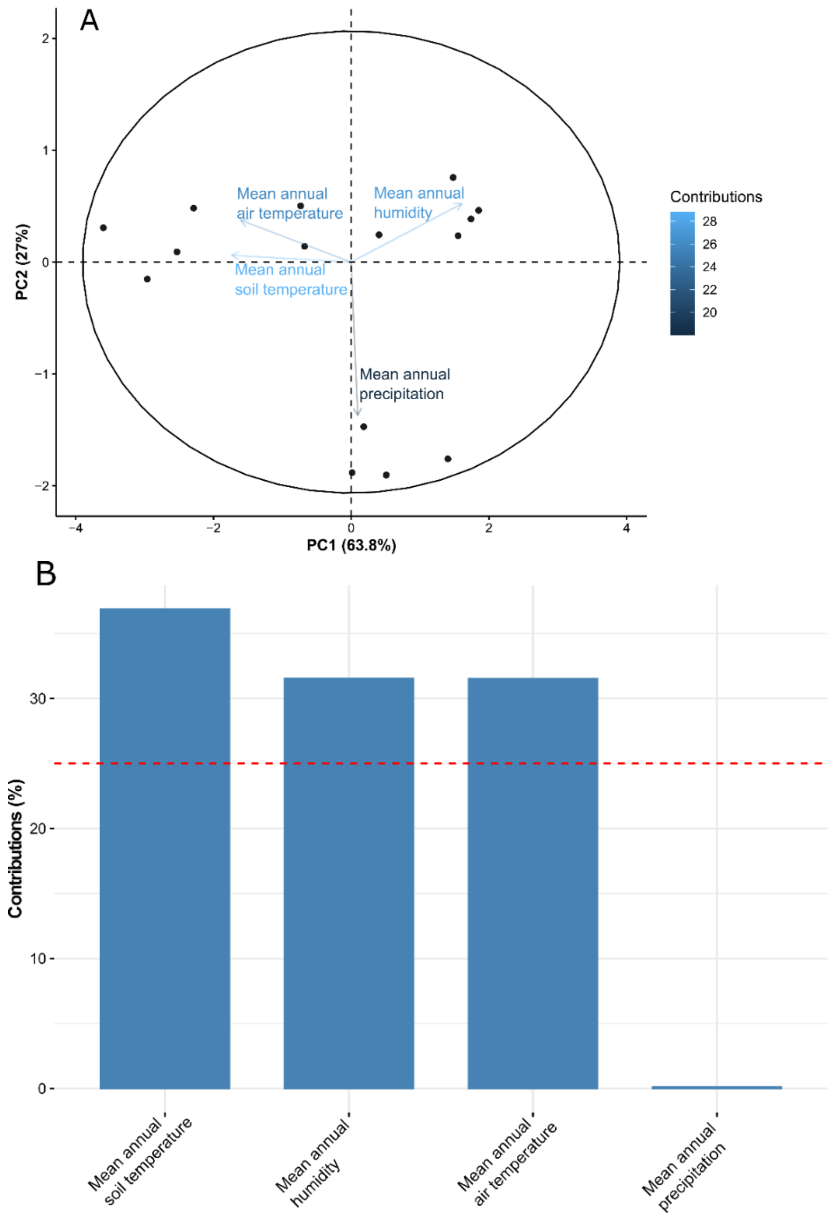

Figure S1. *A) Visualization of principal component 1 (PC1) and principal component 2 (PC2) axes from the principal component analysis (PCA) on the microclimatic variables of the 21 sampled populations. Contributions of each variable is showed on a blue colour gradient, ranging from low contribution (dark blue) to high contribution (light blue). B) Contribution (%) of each microclimatic variable to PC1 axis. Red dashed line indicates the total amount of possible contributions (100%) divided by the number of microclimatic variables (N=4).*

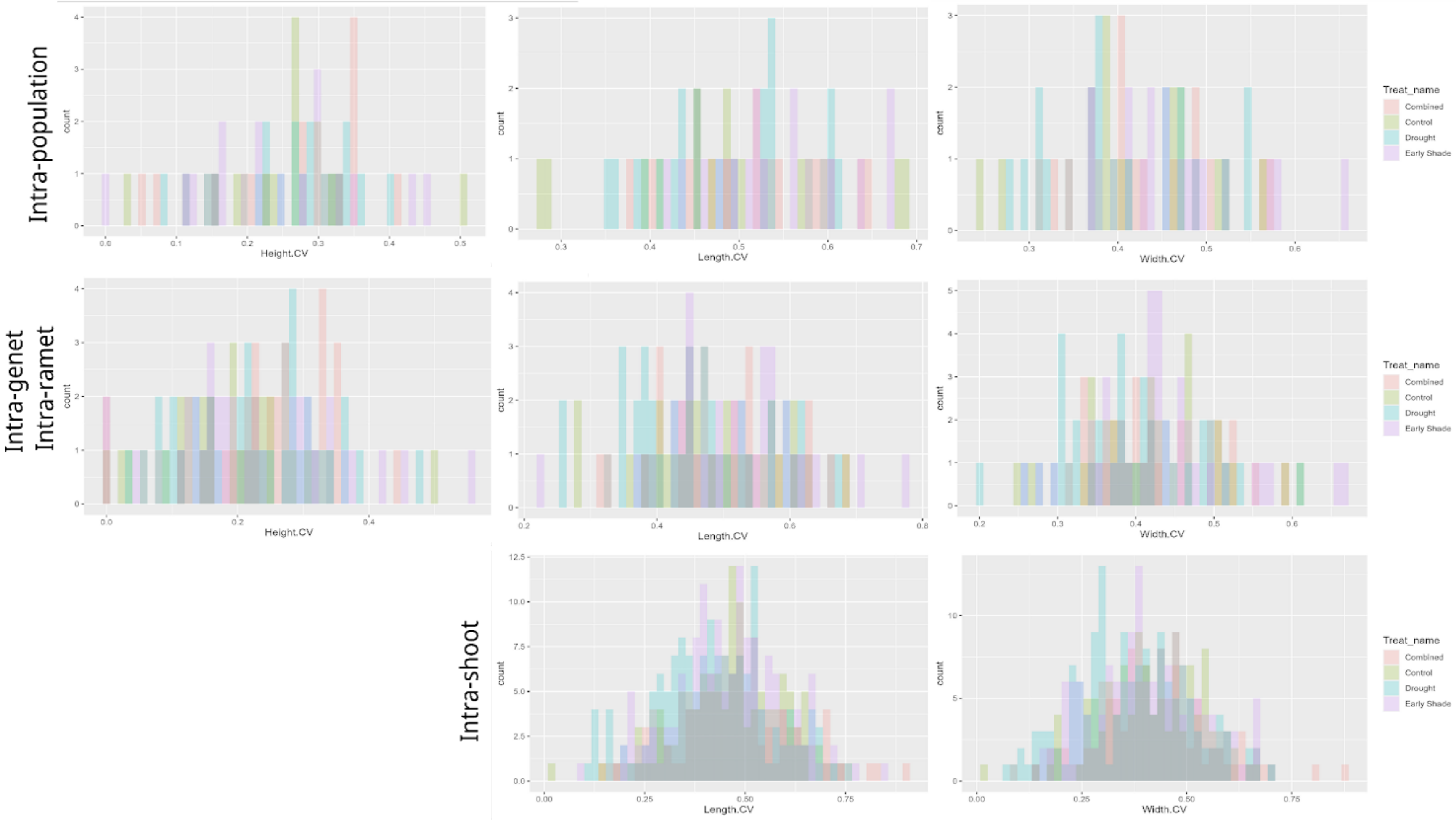

Figure S4: *Histograms for CV in plant height, leaf length and leaf width on each hierarchical level of organisation (intra-population, intra-ramet and intra-shoot) for each treatment combinations (control, drought, shade, and the combined treatments). Due to the experimental design, we cannot distinguish between treatment on the intra-genet level, and this level was therefore not included.*

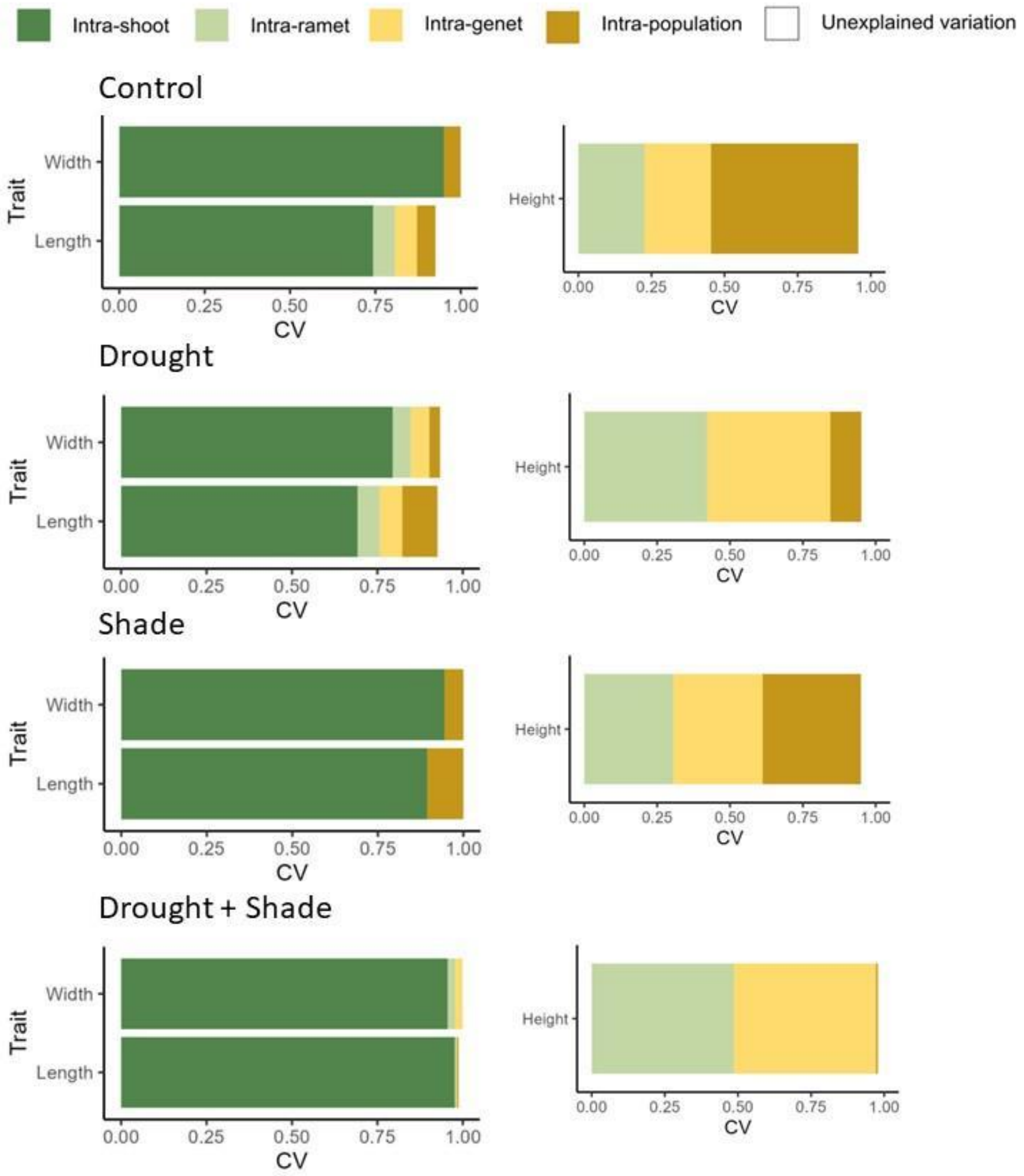

Figure S2: *Proportion of variation in leaf length, leaf width and plant height in Galium odoratum explained by variance partitioning among different levels of organisation: intra-population, intra-genet, intra-ramet, intra-shoot or unexplained variation, for each treatment combination (control, drought, shade and the combined treatment: drought + shade).*