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, 2014; 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 and Simms, 2002; Valladares *et al.*, 2007). However, few studies have looked at genetic adaptation of forest understorey herbs to their local environment (De Frenne *et al.*, 2011; Møller *et al.*, 2022). 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, 1922; Scheepens and 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 and 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 and 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.*, 2011). All species can spread vegetatively to some degree and vary in colonization rates (Brunet *et al.*, 2012).

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, 2021). 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 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 *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, 1922; Scheepens and 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 and 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 and 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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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

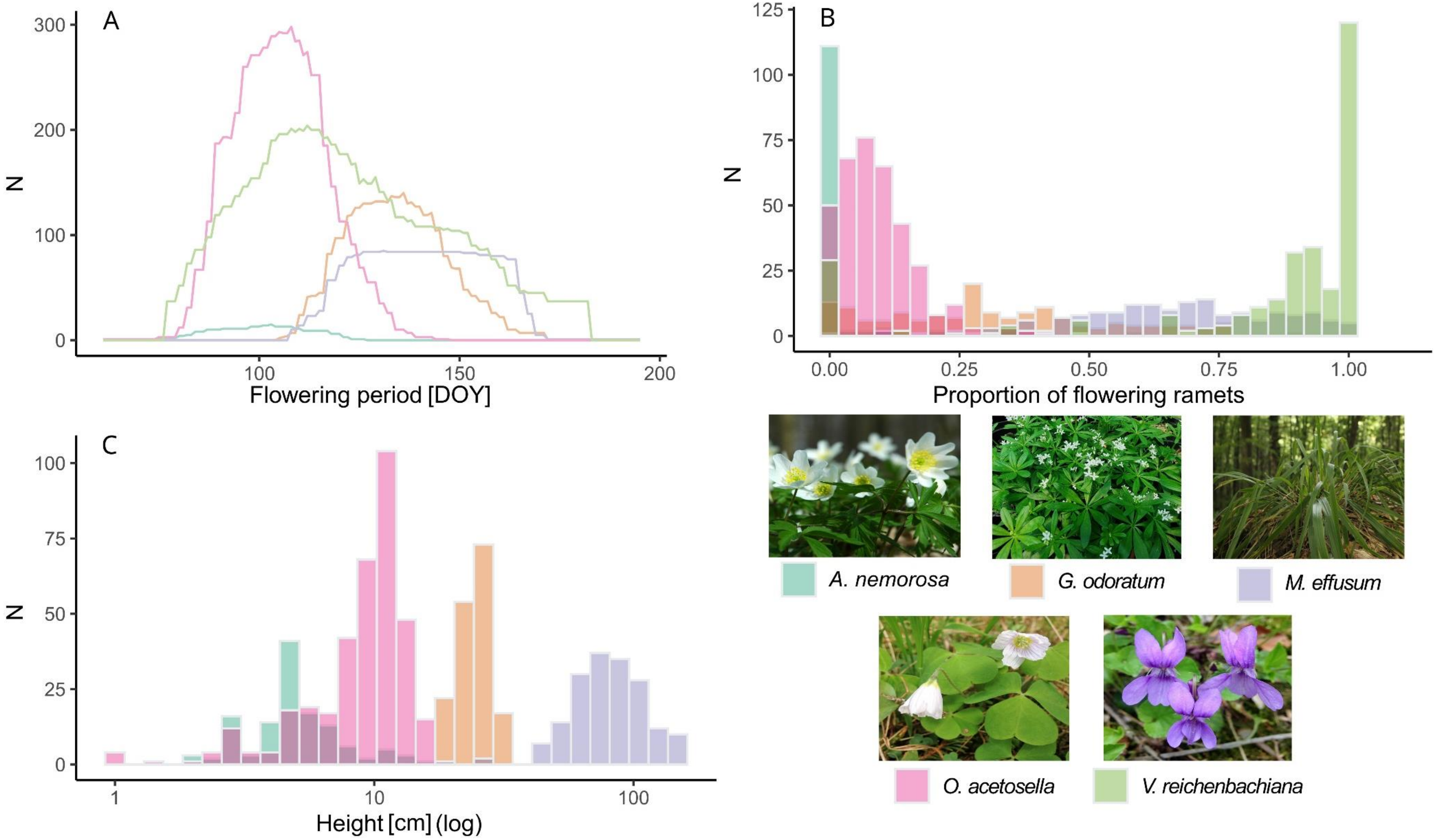

Figure 1. Trait distribution and number of observations (N) for (A) flowering period [DOY], (B) proportion of flowering ramets, and (C) plant height [cm] (x-axis logged) for *Anemone nemorosa*, *Galium odoratum*, *Milium effusum*, *Oxalis acetosella*, and *Viola reichenbachiana*. Photo credits: Franziska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

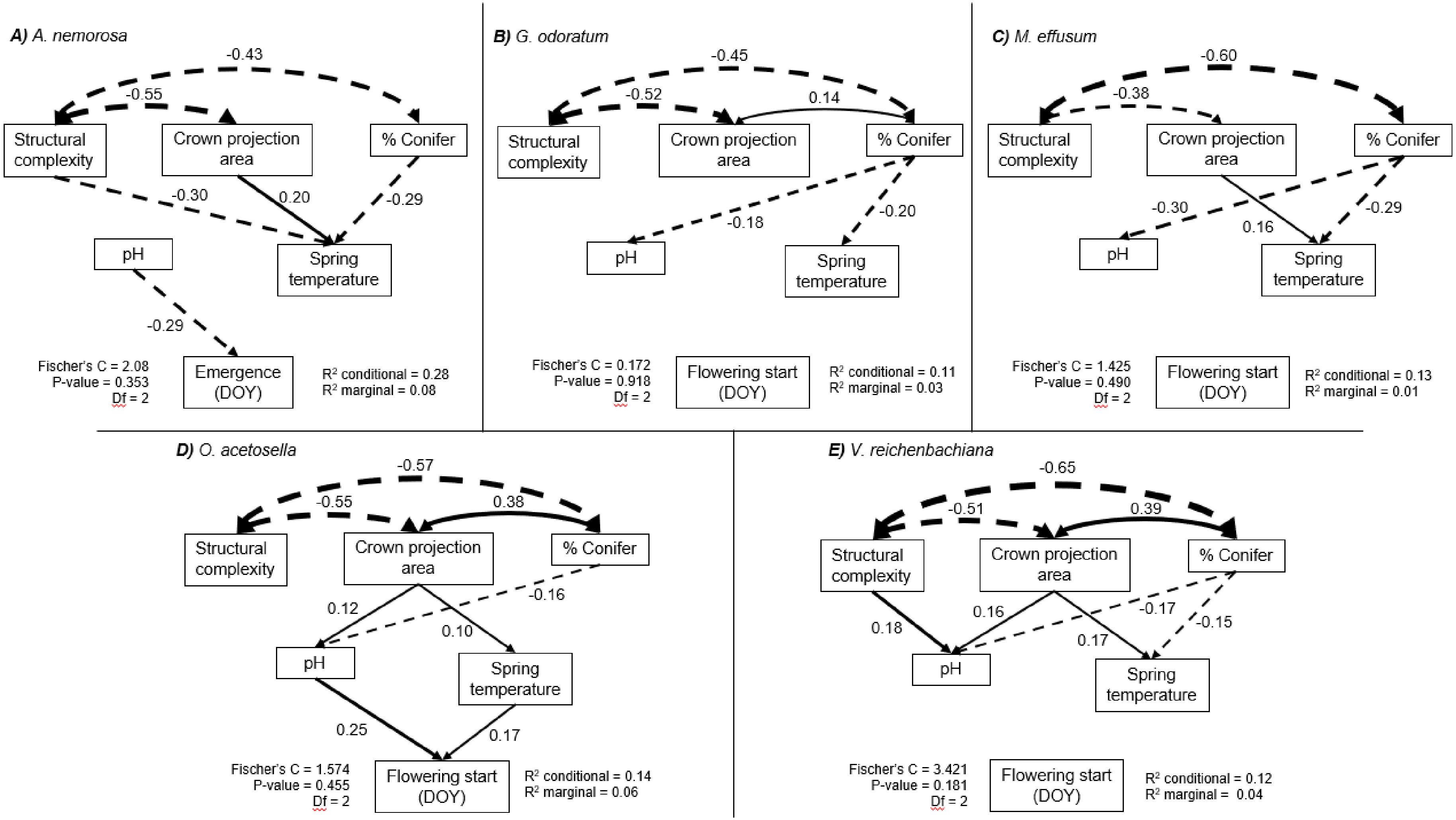

Figure 2. Effects of forest structural attributes and microenvironment of the sites of origin on phenological traits in the common garden. Shown are the results of the piecewise structural equation model (SEM) for direct and indirect relationships among forest characteristics and microclimatic variables, 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).

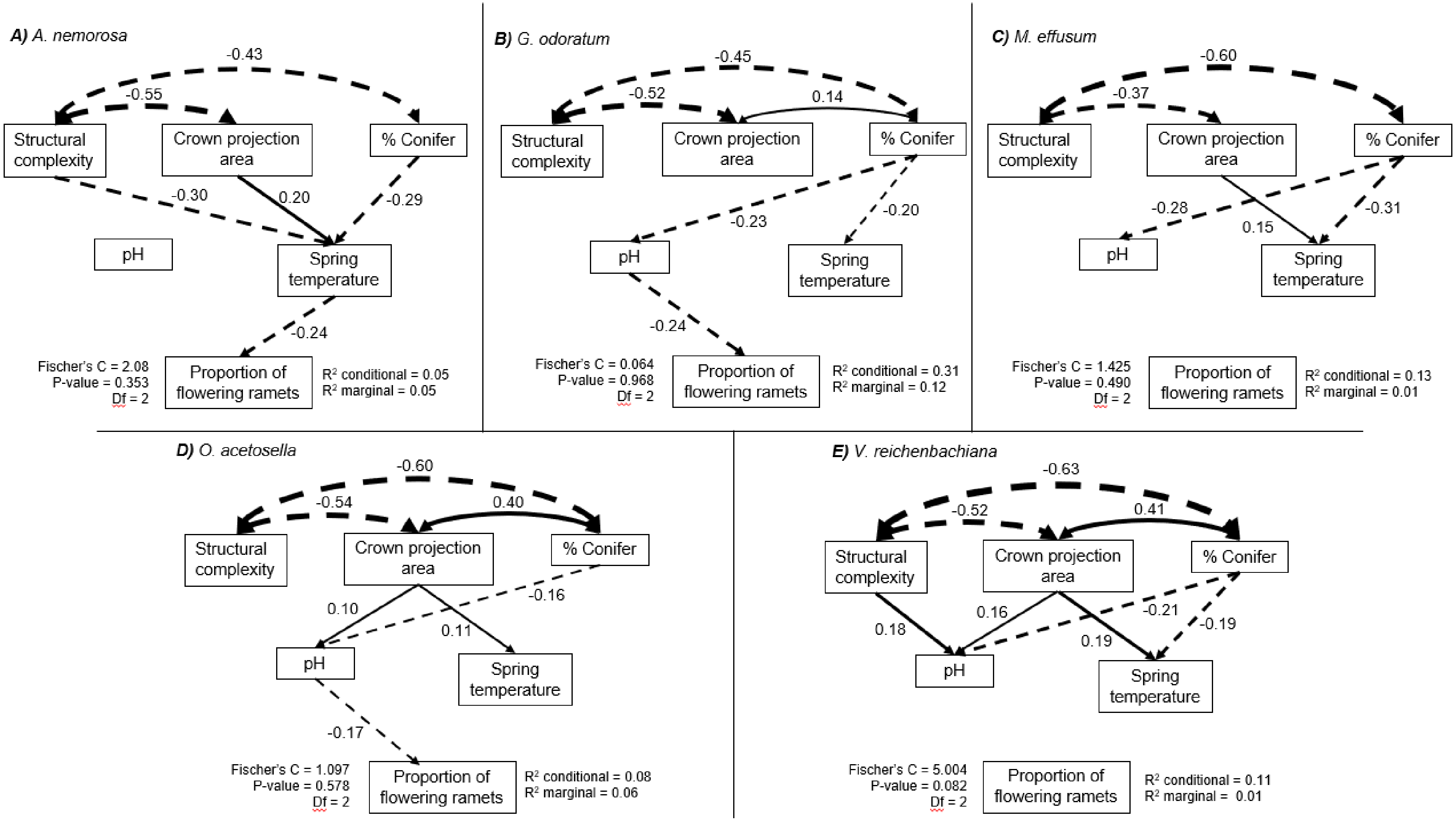

Figure 3. Effects of forest structural attributes and microclimate of the site of origin on flowering proportions in the common garden. Shown are the results of the piecewise structural equation model (SEM) for direct and indirect relationships among forest characteristics and microclimatic variables, 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).

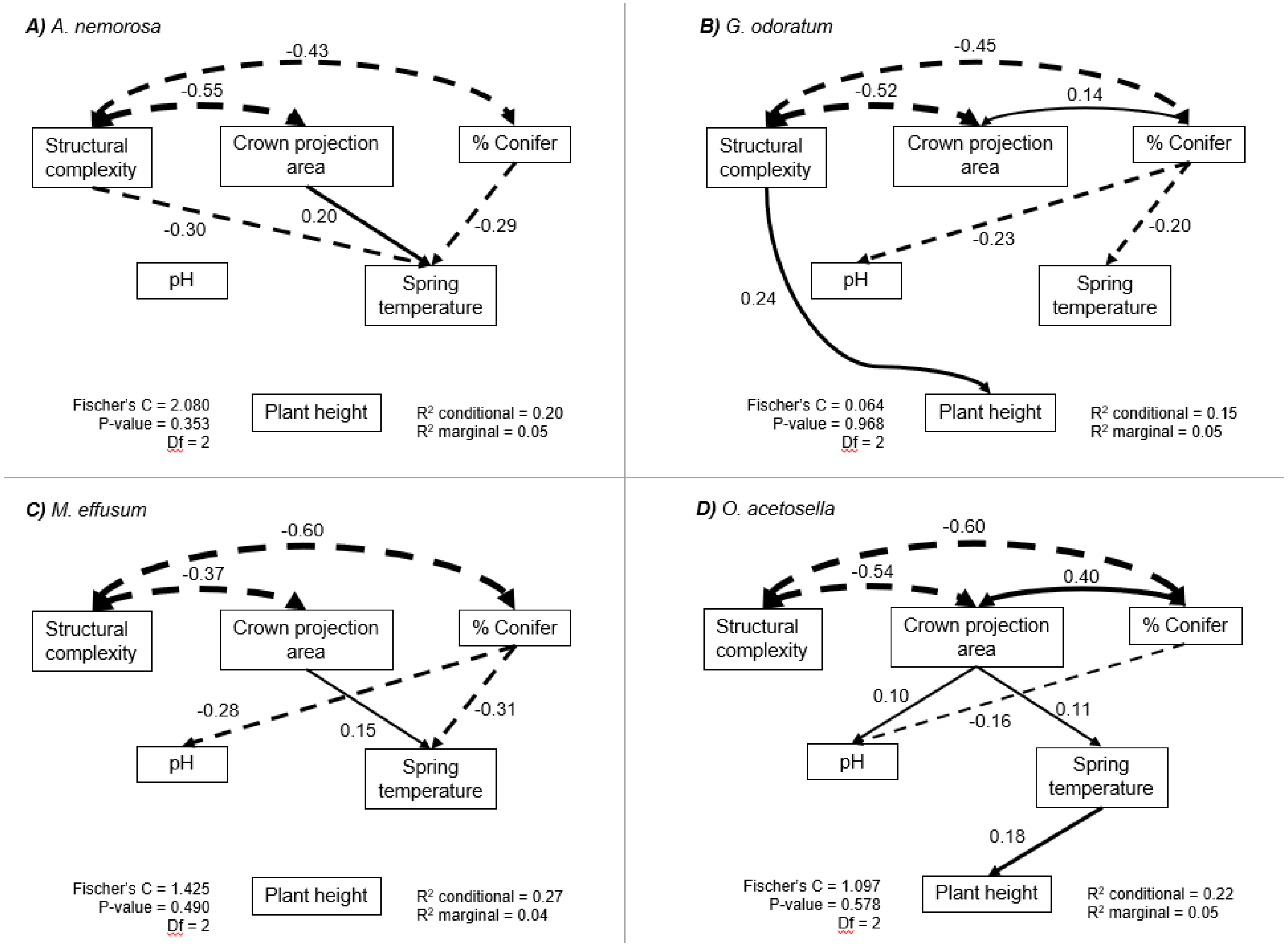

Figure 4. Effects of forest structural attributes and microclimate of the site of origin on plant height in the common garden. Shown are the results of the piecewise structural equation model (SEM) for direct and indirect relationships among forest characteristics and microclimatic variables, and plant height 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).