1 **Title:** Functional trait variation of *Anemone nemorosa* along macro- and microclimatic gradients close to

2 the northern range edge

3 Abstract

4 Climate warming is affecting ecosystems worldwide, and slow-colonizing forest understorey species are 5 particularly vulnerable if they are unable to track climate change. However, species' responses to climatic 6 conditions in terms of growth, reproduction and colonization capacity may vary with the distance to their 7 distribution range edge. Anemone nemorosa is known to be a slow colonizing forest herb dependent on 8 forest cover in the southern and central part of its distribution range, whereas at its northern distribution 9 range (and at higher elevations) it also occurs in open habitats. Here, we investigated the response of plant 10 functional traits of Anemone nemorosa in Central Norway (close to its northern distribution range edge) to 11 a macroclimatic gradient (elevation), two microclimatic gradients (forest density and distance to forest edge), and a competition treatment (removal of neighbouring vegetation). We aimed to identify which 12 13 environmental conditions (light, temperature, soil pH and/or soil organic matter) drive A. nemorosa's 14 responses. In a total of 90 plots, we measured six functional traits of A. nemorosa (plant height, biomass, 15 specific leaf area, seed number, seed mass, and germination percentage). We found stronger variation in 16 environmental conditions along the macroclimatic elevational gradient, than along the microclimatic 17 gradients of forest density and distance to forest edge, and this was also reflected in A. nemorosa's 18 responses. Light availability, in interaction with temperature, was the key environmental variable driving 19 plant performance, while soil conditions were less important. The competition release treatment had a 20 negative effect on A. nemorosa in our study sites, indicating that the facilitative effect of the neighbouring 21 vegetation may be stronger than the competitive effect. Our study suggests that A. nemorosa, close to its 22 northern distribution range edge, has the capacity to cope with climate change through phenotypic 23 responses, and that light and temperature are the key drivers of these responses.

Keywords: Anemone nemorosa, climate change, elevation, forest edge, forest structure, forest
 understorey, functional traits, herb layer, interspecific competition, microclimate

26

28 Introduction

29 Climate warming is affecting a wide array of ecosystems worldwide. Current climate change has induced 30 species range shifts in their geographical distribution (Walther et al., 2002, 2005; Parmesan et al., 2003; 31 Lenoir et al., 2015). Climate change is particularly challenging for sessile organisms such as forest plants 32 that may be unable to track climate change fast enough due to their low migration rates (Malcolm et al., 33 2002; Thomas et al., 2004; Hansen et al., 2006; Engler et al., 2009). For many forest plant species, such slow 34 colonization capacity has been repeatedly demonstrated in forests in modern times (Brunet et al., 1998a, 35 1998b; Cain et al., 1998; Graae et al., 2003; Verheyen et al., 2003). Moreover, colonization into new 36 habitats is strongly limited for many plant species due to the large-scale habitat fragmentation in 37 contemporary landscapes (Honnay, Verheyen, Butaye, et al., 2002; Leimu et al., 2010). As a result, slow-38 colonizing species, including typical ancient forest species, may be particularly vulnerable to (local) 39 extinction under future climate change (Van Der Veken et al., 2004). Given the significance of forest 40 understorey plant species for forest biodiversity and functioning (Gilliam, 2007; Landuyt et al., 2019), it is 41 important to determine to what extent they will be able to respond to future climate change, in terms of 42 acclimation and/or adaptation.

43 However, growth, reproduction and colonization capacities may vary among plant populations within a 44 species and this variation is often demonstrated to depend on prevailing climatic conditions and distance to 45 their distribution range edge. For instance, Helsen et al. (2020) showed how an invasive species increased 46 allocation to reproduction towards northern Europe. Such patterns have also been found for native forest 47 plant species (De Frenne, Brunet, et al., 2011; De Frenne et al., 2012). Laboratory studies have 48 demonstrated that several processes in the reproductive pathway of forest herb species are temperature 49 related (Baskin et al., 1998; Probert, 2000; Finch-Savage et al., 2006), and therefore, it is likely that 50 reproductive efficiency (e.g. seed production, seed mass or germination rate) will change in response to 51 rising temperatures. For instance, the germinability of slow-colonizing forest species increased with 52 warming (Graae et al., 2009). Also, vegetative growth (plant height, aboveground biomass) and 53 reproductive success (seed mass, seed germination and germinable seed output) of Anemone nemorosa, a 54 typical forest understorey species, benefited from higher temperatures, while specific leaf area of the

species decreased with warming (De Frenne, Graae, et al., 2011). In an experimental setup including several temperate forest understorey species, plants grew taller in response to both increased temperature and light availability, while specific leaf area (SLA) showed contrasting responses to increasing temperature and light for different understorey species (Blondeel et al., 2020). Understanding how functional traits and performance vary with climatic gradients may help us understand and predict species population dynamics in response to climate change.

61 This understanding of the impact of climatic conditions on plant performance is further complicated by a 62 partial decoupling between the macroclimate and the microclimate. Microclimatic buffering occurs when 63 the environmental conditions experienced by the understorey at the forest floor differ from the 64 macroclimate outside forests. Through shading and evapotranspiration, the tree canopy strongly influences 65 the local thermal conditions at the forest floor, creating a microclimate (a local climate differing from free-66 air temperatures). Several studies have investigated the microclimatic buffering capacity of forests (Geiger 67 et al., 2012; Frey et al., 2016; Lenoir et al., 2017; Bramer et al., 2018; De Frenne et al., 2021). In a global 68 meta-analysis, De Frenne et al. (2019) showed that tree canopies function as a thermal insulator, cooling 69 the understorey when macroclimatic temperatures are high (in summer), and warming the understorey 70 when macroclimatic temperatures are low (in winter). The level of microclimatic buffering depends on the 71 canopy structure and composition (Zellweger et al., 2019), which can create climatic gradients within 72 forests (e.g. from the edge to the core) (Honnay, Verheyen, & Hermy, 2002; Geiger et al., 2012). Moreover, 73 microclimatic buffering can differ with latitude. Graae et al. (2012) found that summer temperatures at 74 weather stations and below the forest canopy differed less towards higher latitudes, i.e. microclimatic buffering in summer decreased with latitude. 75

Here we study the widespread European understorey forest herb Anemone nemorosa L. (Ranunculaceae) in central Norway, close to its northern distribution range edge (further on referred to as 'northern limit'). In Western and Central Europe, A. nemorosa is a model species for slow colonizing herbaceous forest plants (Hermy, 1994; Brunet et al., 1998b). It is a spring flowering geophyte, characteristic of ancient broadleaved forests. Interestingly, the species appears less confined to ancient forests close to its northern limit in both Norway and Sweden where the species commonly occurs in disturbed and grass-dominated habitats

82 outside forests (https://www.artsdatabanken.no; http://linnaeus.nrm.se/flora). In Central Europe, the species can also be found outside of forests, but mainly at higher elevations (e.g. in montane meadows; 83 84 Leuschner et al., 2017). The populations found close to their northern limit may have better colonization 85 capacity and/or opportunities in a warming climate. We, however, do not know if the fact that A. nemorosa 86 appears to colonize more habitats relates to a wider realised temperature niche or to a more favourable 87 light regime (longer photoperiods in the growing season) at higher latitudes. We evaluated six functional 88 traits of natural populations of A. nemorosa, along a macroclimate gradient (i.e. elevation) and two 89 microclimate gradients (i.e. forest density and distance to forest edge). In addition, we evaluated the effect 90 of competition with neighboring vegetation along these gradients. Three traits were related to vegetative 91 growth: (i) specific leaf area (SLA) is related to the plant's resource-use strategy and represents a trade-off 92 between photosynthetic rate (high SLA) and leaf longevity (low SLA) (Wright et al., 2004; Cubino et al., 93 2021); (ii) plant height corresponds with the ability to pre-empt light resources and disperse seeds (Díaz et 94 al., 2016); and (iii) aboveground biomass is an approximation of plant productivity. Three traits were 95 related to reproductive success: (i) seed mass and (ii) seed number reflect the trade-off between dispersal 96 and colonization ability (larger number of small seeds) on the one hand, and seedling survival and 97 competitiveness (smaller number of large seeds) on the other hand (Díaz et al., 2016); and (iii) germination 98 rate reflects the resource competitive ability of a plant (Zhang et al., 2018). To date, research on the effects 99 of temperature on population dynamics of A. nemorosa focused especially on macroclimate effects along 100 broad latitudinal and elevational gradients (Mondoni et al., 2008; Graae et al., 2009; De Frenne, Graae, et 101 al., 2011).

- 102 We specifically asked the following research questions:
- 103 (i) How do (vegetative and reproductive) functional traits of *A. nemorosa* respond to the climatic
 104 gradients and the competition treatment;

105 (ii) what are the key environmental factors (light, temperature and/or soil conditions) that drive
106 these responses.

- 107 A better understanding of the species' response to different climatic conditions is highly relevant for
- 108 predictions of its distribution range under future climate change. In addition, our findings will increase our
- 109 understanding of the frequent occurrence of *A. nemorosa* in more open habitats in Norway and Sweden
- (i.e. close to its northern limit), in contrast to its confinement to closed forests elsewhere in Europe.
- 111 Moreover, it can provide insights into what factors are limiting its expansion into new areas under future
- 112 climate change.

114 Methods

115 Study species

116 Anemone nemorosa L. (Ranunculaceae) is one of the most widespread herb species in ancient European 117 woodlands (Shirreffs, 1985; Brunet et al., 1998b). Ancient woodland refers to land which has remained 118 wooded since a certain threshold date (e.g. 1600) (Peterken et al., 1984). In contrast to primary forest, it 119 can also refer to managed forests. Anemone nemorosa is an early flowering perennial forest plant, with a 120 geographical distribution range from 38 °N in southern Europe to 67 °N in Norway (Hultén et al., 1986). It 121 grows on a wide range of soil types, both in terms of soil moisture and soil pH (pH range of 3.5 – 8.0), and 122 can persist in open areas (Shirreffs, 1985). Shoots appear from February to May (depending on latitude) 123 and white flowers (one per shoot; sometimes pink) appear about two weeks later (De Frenne et al., 2010). 124 Flowers are mostly self-incompatible and pollinated by insects, but selfing can occur (Müller et al., 2000). 125 The species usually produces 10-30 achenes (henceforth referred to as seeds) per year and per flowering 126 ramet (henceforth referred to as individual) (De Frenne et al., 2010). Vegetative growth through rhizomes is 127 very slow, and it is assumed that seeds are the most important dispersal organ accounting for population 128 persistence and spread (Brunet et al., 1998b; Müller et al., 2000; Stehlik et al., 2000). Seeds are mainly 129 gravity-dispersed, but myrmecochory and slug-dispersal can occur as well (Delatte et al., 2008; Türke et al., 130 2010). In addition, roe deer are known to feed on flowering shoots of A. nemorosa and may also transport 131 seeds over longer distances (Brunet et al., 1998a; Mårell et al., 2009).

132 Study region and set up

We studied plant functional traits of *A. nemorosa* in Central Norway along three macro- and microclimatic gradients: elevation, forest density, and distance to the forest edge. In total, we selected nine forest stands: three at low elevation (27 - 42 m.a.s.l.), three at intermediate elevation (221 - 283 m.a.s.l.) and three at high elevation (675 - 681 m.a.s.l., approaching the tree line in the area). Latitudes of the stands ranged from 62.56°N to 63.50°N, which is close to the northern limit of *A. nemorosa* (exact locations are provided in SI Appendix 1). All forests were ancient forests (i.e. continuously forested and not converted to another land use since the oldest available land use maps typically at least 150–300 years old), with a tree layer

consisting of *Alnus glutinosa, Sorbus aucuparia, Alnus incana, Ulmus glabra, Betula pubescens* and *Pinus sylvestris* (SI Appendix 1). The most common herb species were *Anemone nemorosa* and *Oxalis acetosella*.
The mean monthly temperature in May 2018 was 12.9°C, 12.7°C and 12°C at the low, middle and high
elevations, respectively. In June 2018, this was 11.0°C, 11.6°C and 10.9°C (climatic data from Yr, delivered
by the Norwegian Meteorological Institute and NRK).

145 At each elevation, the three selected stands differed in canopy density (see also Govaert et al., 2020;

146 Meeussen et al., 2020). We defined 'dense forests' as vertically complex with a well-developed shrub layer.

147 'Intermediate forests' had a sparse or absent shrub layer. 'Open forests' were structurally simple with no

shrub and subdominant tree layer. We confirmed this classification through available measurements of

structural canopy characteristics. The mean canopy closure (measured with a densiometer; Jennings et al.,

150 1999; Baudry et al., 2014) was 96.0 % (± 2.8 s.d.) in dense forests, 93.4 % (± 5.9 s.d.) in intermediate forests,

and 87.2 % (± 11.7 s.d.) in open forests. Mean values of plant area index (the total of the one-sided area of
 woody and non-woody biomass per unit of surface area, determined with terrestrial laser scanning; see

153 Meeussen et al. (2020) for further details) were 4.2 (± 0.8 s.d.), 4.1 (± 1.4 s.d.) and 2.8 (± 1.4 s.d.), in dense,

154 intermediate and open forests, respectively (SI Appendix 2).

155 In each forest stand, we established a 100 m long transect from the southern forest edge towards the 156 forest interior. The studied edges were bordered by arable land with barley at low elevation, and grassland 157 at intermediate and high elevation (SI Appendix 1). In each transect, we established five 20 cm x 40 cm study plots at an exponentially increasing distance perpendicular to the forest edge, because of the 158 159 exponential change in microclimatic conditions close to the edge (Chen et al., 1999). We set out plots at 160 1.5, 4.5, 12.5, 36.5 and 99.5 m from the edge (defined as the outermost line of tree trunks). Plots were not 161 always located exactly on the transect line, but their location was chosen to maximize the occurrence of A. 162 nemorosa, staying within a distance of max. 10 m perpendicular to the transect line. All plots were at least 163 100 m away from any other forest edge.

In addition to the climatic gradients, we investigated the effect of interspecific competition. Therefore,
each plot was divided in two subplots (20 cm x 20 cm), and in one of these subplots, all bryophytes and all

herbaceous species except *A. nemorosa* were removed by clipping. This was repeated weekly, to minimize
competition during the entire growing period of *A. nemorosa*. These other species are expected to compete
with *A. nemorosa* for light, water and nutrients (Navas et al., 2009), and may also influence *A. nemorosa*through altering the soil temperature. The applied competition release treatment can provide further
insight into the importance of these competitors and the microclimatic feedbacks for the investigated
functional traits of *A. nemorosa*.

172 Close to each forest transect, we also established a study plot containing *A. nemorosa* outside of the forest. 173 The distance of this sixth plot to the forest edge differed among sites, depending on the occurrence of 174 available natural vegetation with *A. nemorosa*, and varied between 1 and 30 m. These plots were typically 175 situated in a grassland adjacent to the forest, or in small grass strips as buffer areas between forests and 176 arable fields. In these nine plots outside the forest, we collected the same data and applied the same 177 competition treatment as for the plots along the forest transect.

178 Microclimate and soil data

179 In the centre of each subplot, at 10 cm above the soil surface, we installed a Hobo Pendant 180 temperature/light data logger attached to a short steel stick, recording temperature (°C) and light intensity (lux; spectrum: 150 - 1200 nm) every hour from the 22th of May until the 13th of June, i.e. the flowering 181 182 period of A. nemorosa in the region. We used daily minimum and maximum temperatures to calculate the 183 number of growing degree hours (GDH) above a base temperature (5°C) from the start of the 184 measurements until the trait collection dates as a measure of accumulated temperature (Lindsey et al., 185 1956). The GDH-concept is often used as a substitute for temperature because of the demonstrated 186 relationship between plant development and GDH (Diekmann, 1996; De Frenne et al., 2010). As a measure for light intensity in each subplot, we calculated the mean of the hourly-recorded light intensity values over 187 the same period as applied for GDH calculations. 188

189 In each plot, we collected a soil sample from 0-15 cm depth after removing the litter layer. All soil samples

190 were dried in the oven for 72 hours at 60°C, ground and sieved over a 2-mm mesh. We analysed soil

samples for pH-CaCl₂ by shaking a mixture of 10 g soil in 30 ml 0.01 M CaCl₂ and measuring the pH of the

supernatant solution after 30 minutes of settling (Schofield et al., 1995). Total soil organic matter (SOM)

193 was measured as loss on ignition by heating the samples at 550°C for 6 hours (Konare et al., 2010).

194 Traits of Anemone nemorosa

We measured six traits (belonging to both morpho-, physio- and phenological traits and performance traits
(sensu Violle et al., 2007)) on five individuals per subplot. Three traits were related to sexual reproduction:
number of seeds, seed mass and germination percentage. In addition, we measured three vegetative traits:
height, specific leaf area (SLA), and aboveground biomass.

199 To collect seeds, we installed small permeable and breathable bags around the flower of each individual 200 after pollination, respectively on 1st June, 18th May and 22nd May of 2018 in the low, middle and high 201 elevation sites. The bags were installed after anthesis and pollination as indicated by petal abscission. The seeds were collected on 13th, 14th and 15th June 2018, in the low, middle and high elevation sites 202 203 respectively. Seeds were air-dried at room temperature for one week. We counted the number of seeds, 204 and weighed all seeds of each individual. The mean seed mass was then calculated as the ratio of the total 205 seed mass divided by the number of seeds for each individual. Subsequently, the seeds were placed on 206 filter paper into Petri dishes (per individual) with distilled water at room temperature (+/- 20°C) for eight 207 weeks. During this time, distilled water was applied to keep the filter paper moist. After eight weeks, all 208 seeds were treated with cold stratification in a growth chamber at 10°C and darkness for six weeks 209 (Mondoni et al., 2008). Germination was recorded as radicle appearance (> 1mm) every 3 days during the 210 six weeks of cold stratification. Germination percentage was calculated for each individual.

Simultaneously with the collection of the seed bags, we harvested the five plant individuals in each subplot at the base of the stem and stored them fresh and wet in plastic bags. Within 72 hours, we measured the stretched height of the plant from the base of the stem to the highest photosynthetic expanded tissues, and separated a healthy leaf from each individual to measure its fresh weight on a balance and scanned for later area measurement. Subsequently, the plant and leaf were dried for 72 hours at 60°C and weighed again to determine dry leaf mass and plant biomass. Specific leaf area (SLA) was obtained by dividing the fresh leaf area by its oven dried mass.

- 218 In 84 out of 90 subplots, five A. nemorosa individuals were present. In two subplots (within the same plot),
- all individuals disappeared after plot installation (probably due to herbivory), and in four subplots, only one
- to three individuals were present. In total, 428 individuals were recorded, of which 304 developed seeds.

221 In addition to the analyses along the forest transect, we performed an additional analysis to compare plant

222 performance of *A. nemorosa* occurring in the forest interior to the performance of individuals in the control

plot established outside of the forest in a grassland. These results are shown in SI Appendix 3.

224

225 Data analyses

We performed statistical analyses in R version 3.6.2 (R Core Team, 2019), using the packages *Ime4* (Bates et al., 2015), *ImerTest* (Kuznetsova et al., 2019) and *MuMIn* (Barton, 2019).

228 First, we assessed the impact of the macro- and microclimatic gradients (elevation, forest density and

distance to the edge) and the competition treatment on the environmental conditions in each (sub)plot.

230 The effects on two microclimate-related response variables, growing degree hours (GDH) and light intensity

- 231 (eq. 1), and on two soil-related response variables, soil organic matter (SOM) and soil pH (eq. 2), were
- tested using linear mixed models:

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233 Microclimatic response ~ (elevation + density + distance to edge + competition)<sup>2</sup> + (1|transect/plot) (Eq. 1)
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234 Soil response ~ (elevation + density + distance to edge)² + (1 | transect) (Eq. 2)

²³⁵ '^{2'} indicates that all two-way interactions between predictor variables were included in the model.

For the soil variables, competition was not included as a predictor variable, as we did not expect an
immediate effect of the competition on SOM and soil pH and only took one soil sample per plot. Elevation
was included as a factor with three levels: high, intermediate and low elevation. Forest density was also
included as a factor with three levels: dense, intermediate and open forest. Distance to the edge was
included as a continuous variable, and was log-transformed to account for the exponential pattern in the
plot-distribution along the transect. In addition, this variable was scaled to unit variance and mean zero.
Competition was included as a factor with two levels, i.e. with and without competition. GDH, mean light

intensity and SOM were log-transformed to obtain normal distribution of these response variables. To
account for the hierarchical structure of the data, transect was included as a random effect with varied
intercepts only, and in the case of the microclimatic variables, also the plot was included as a random
effect, nested within transect.

Second, we used linear mixed-effect models, to explore how each of the six plant functional traits
responded to (i) the design variables (i.e. elevation, forest density, distance to edge and competition), and
(ii) the measured environmental conditions (i.e. GDH, light, soil pH and SOM). We also tested each two-way
interaction between the predictors. To account for the hierarchical structure of the data, transect and plot
were again included as random effects with varied intercepts only, with plot nested within transect.

Eq. 3 represents the structure of the model with the design variables. Eq. 4 represents the structure of themodel with the environmental conditions.

²⁵⁶ ²² indicates that all two-way interactions between predictor variables were included in the model. The predictor variables in eq. 3 are the same as in eq. 1, and are described above. In eq. 4, all predictor variables were continuous and scaled to unit variance and mean zero. GDH, mean light intensity and SOM were log-transformed to obtain normal distribution. Biomass and seed mass were log-transformed to obtain normal distribution of these response variables. For the response variable 'seed number', we applied a Poisson distribution. For the response variable 'germination percentage', we applied a binomial distribution (Zuur et al., 2009).

263

We calculated variance inflation factors (VIFs) to assess multicollinearity of the predictor variables in all models. This resulted in the exclusion of the interaction term elevation x forest density in the models with the design variables as predictors (eq. 1-3), because VIF was > 40. All other predictor variables in each set of models had VIFs < 10. We found no clear patterns in the residuals for each model, based on graphical evaluation (Zuur et al., 2009). For each model, we calculated the marginal and conditional *R*², representing

- the variance explained by fixed factors and the variance explained by both fixed and random factors,
- 270 respectively (*MuMIn* package; Nakagawa et al., 2013).

275 Results

277

276 Environmental conditions

278 lowest GDH at low elevation, and the highest GDH at intermediate elevation. In addition, we found a 279 significant interactive effect between distance to edge and forest density (p = 0.012) on GDH: in open and 280 dense forests, GDH decreased towards the forest interior, while in intermediate forest types, GDH 281 increased towards the forest interior (Fig. 1A; SI Appendix 4). 282 Light intensity in each subplot also depended on elevation (p = 0.004), with highest light levels at high 283 elevation and lowest light levels at low elevation. Similar to GDH, we found a significant interactive effect 284 between distance to edge and forest density (p < 0.001) on light: in open forests, light levels decreased 285 towards the forest interior, while in dense forests, light levels did not change much along the transect, and 286 in intermediate forests, light levels increased towards the forest interior (Fig. 1B; SI Appendix 4). 287 Soil pH was higher towards lower elevations (p = 0.022) and closer to the forest edge (p = 0.020). Soil 288 organic matter was not significantly correlated with any of the design variables (SI Appendix 4; SI Appendix 289 5).

The temperature in each subplot (expressed as GDH) depended on the elevation (p < 0.001), with the



291

290

Figure 1. Environmental conditions along the forest transect. Mean values and standard error bars are shown for
 growing degree hours (GDH) (a) and light levels (b) at the five locations along the forest transect (note that data of the
 five plots along the transect are distributed evenly on the x-axis, but the distance is in fact exponentially increasing).
 Colours indicate the three different elevational levels. Different symbols indicate the three forest density levels.

296

297 Functional trait response to design variables

- 298 We found a significant negative effect of high elevation on SLA (p = 0.008). The removal of competition had
- a negative effect on plant biomass (p = 0.027). We also found a significant interactive effect between
- 300 distance to the forest edge and forest density on both SLA and plant height. While SLA increased towards
- 301 the forest interior in both open and closed forests, it slightly decreased in forests with intermediate density
- 302 (p = 0.038). Similarly, while plant height did not change towards the forest interior in open and closed
- forests, it decreased in intermediate forests (p = 0.049) (Fig. 2A; SI Appendix 6.
- 304 Germination rate was significantly higher at mid elevation (p = 0.009) and high elevation (p = 0.002) than at
- low elevation. The removal of competition negatively affected seed mass (p = 0.047). In addition,

306 competition removal positively affected seed number, but only at the high elevations (interactive effect; p = 0.003). At high elevations, seed number decreased towards the forest interior, while it increased at low and 307 308 mid elevations (interactive effect; p = 0.007). The effect of competition removal on germination rate was 309 positive in closed and intermediate forests, but negative in open forests (interactive effect; p = 0.002) (Fig. 310 2B; SI Appendix 6).

(a)



312



318 model. See SI Appendix 6 for table with full model results.

319 Functional trait responses to environmental variables

Biomass

 $(R^2m = 0.06; R^2c = 0.29)$

We found a negative effect of light on SLA (p = 0.017). Furthermore, we found a significant interactive effect (p = 0.006) between light and GDH on plant biomass: the positive effect of light on plant biomass was stronger at low levels of GDH, and weak at high levels of GDH (**Fig. 3A**; SI Appendix 7; SI Appendix 10). For seed mass and seed number, we found an interactive effect between light and GDH (p = 0.039 and p < 0.001, resp.), which was similar to the findings for plant biomass: the positive effect of light decreases with increasing GDH. For germination rate, the effect of light depended on soil pH, with positive effects at high soil pH, and negative effects at low soil pH (p = 0.006) (**Fig. 3B**; SI Appendix 7; SI Appendix 10).

Plant height

 $(R^2m = 0.07; R^2c = 0.57)$

SLA

 $(R^2m = 0.16; R^2c = 0.38)$

GDF

(a)

327

328



329 Figure 3. Model results for the functional traits as function of the environmental variables. (a) traits related to

330 vegetative growth; (b) traits related to reproductive success. Estimates and 95% confidence intervals for each

- explanatory variable in the model are shown. Non-significant effects (with confidence intervals including zero) are
- indicated with grey circles. Significant effects are highlighted as black traingles. Marginal R² (R²m) and conditional R²
- 333 (R²c) are indicated for each model. GDH = growing degree hours; SOM = soil organic matter. See SI Appendix 7 for
- table with full model results.
- 335

337 Discussion

338 Our study adds evidence to the idea that A. nemorosa has the capacity to cope with climate change 339 through phenotypic responses (Shirreffs, 1985). Environmental conditions varied more strongly along the 340 macroclimatic elevation gradient, than along the microclimatic gradients of forest density and distance to 341 the forest edge, and this was reflected in the response of the plant functional traits, which were mostly 342 affected by the elevational gradient. Light availability was the key environmental variable behind this 343 observation. There was also an interactive effect between light and temperature such that the positive 344 effect of light on plant performance (biomass, seed mass and seed number) was stronger at lower 345 temperatures than at higher temperatures. The competition release treatment had a negative effect on 346 plant performance (biomass, seed mass, germination rate), suggesting that in our study sites, the 347 facilitative effect of the neighbouring vegetation (such as creating a favourable soil microclimate) is 348 stronger than the competitive effect on A. nemorosa.

Given the limited importance of soil conditions to explain variation in plant functional traits, we will focus
 our discussion below on light and temperature as environmental drivers. The variation of soil conditions
 across climatic gradients and the impact of soil pH on germination rate are briefly discussed in SI Appendix
 5.

353 Environmental conditions across climatic gradients

354 Overall, the environmental conditions in our study plots were mostly determined by the elevational 355 gradient. Light availability at the forest floor increased towards higher elevations, which is probably mainly 356 the effect of differences in tree species composition and forest structure. Measurements of plant area 357 index, based on terrestrial laser scanning, confirmed that forest density decreased towards higher 358 elevations (see SI Appendix 11 and Meeussen et al. (2020) for further details). At the highest elevation, all 359 sites were dominated by Betula pubescens, which is characterized by a sparse canopy (Beck et al., 2016) 360 with a low shade-casting ability (Ellenberg, 1996), while at the lower elevation sites, forests were 361 dominated by Ulmus glabra and Alnus incana with a higher shade-casting ability.

362 In general, macroclimate temperature is expected to decrease with elevation, at an average lapse rate of -0.55°C (100 m)⁻¹ (Körner, 2007). Such climatic gradients offer excellent opportunities to assess long-term 363 364 ecosystem responses to climate change (using an indirect space-for-time substitution; Fukami et al., 2005). 365 However, in our study region, although the mean annual temperature confirmed this generally expected 366 trend (SI Appendix 1), we did not observe a clear decreasing trend in GDH towards higher elevation during our study period (22nd May – 13th June), as we found the lowest GDH-values at the low elevation sites. This 367 368 stresses the importance to measure in situ microclimates, and certainly so in forest ecological studies and 369 highly heterogenous topography. Many studies have indeed demonstrated that perfect gradients of 370 temperature with elevation are hard to find due to much local variation in topography, vegetation cover, 371 local hydrology and shade and wind effects (Körner, 2003; Nyakatya et al., 2008; Scherrer et al., 2010; 372 Wundram et al., 2010; Suggitt et al., 2011; De Frenne et al., 2021). Our unexpected temperature findings 373 emphasize the need for using relevant temperature data, at the scale experienced by organisms, 374 incorporating microclimatic variation, in climate impact studies (Graae et al., 2012). In this study, a similar 375 unexpected trend in GDH with elevation (i.e. highest GDH at intermediate elevation and lowest GDH at low 376 elevation) was measured in the control plots that were established outside of the forest (described in SI 377 Appendix 3). This suggests that local differences in shading by the canopy cover is indeed not the only 378 driver of the unexpected elevational temperature gradients, and that very local weather differences have 379 occurred during the study period.

380 We found differences in local light and temperature conditions (i.e. the microclimate) along the forest 381 transect from the edge to the core. In general, it is expected that microclimatic buffering increases towards 382 the forest core, where lower light levels and GDH occur (Chen et al., 1999; Honnay, Verheyen, & Hermy, 383 2002; Geiger et al., 2012; Meeussen et al., 2020). In open forests, our observations confirmed this 384 expectation, as light and GDH decreased towards the forest core (SI Appendix 4). In dense forests, only GDH 385 decreased towards the forest core, whereas light levels at the forest floor did not change along the forest 386 transect, suggesting that a dense canopy might already reduce light levels considerably from the forest 387 edge onwards. The most unexpected finding, however, was that both GDH and light increased towards the 388 forest interior in forests with intermediate density. We confirmed that this unexpected finding was not

driven by one study site only: in the intermediate forest transects, at all three elevations, light and GDH
were higher in the forest core than in the edge (SI Appendix 9). This finding is likely driven by the
decreasing canopy closure (measured with a densiometer, SI Appendix 2) towards the forest interior that
was observed in the intermediate forests.

393 Plant performance across climatic gradients

Plant performance was mainly driven by the elevational gradient, while the microclimatic gradient (forest
density and distance to forest edge) was less important to explain the variation in plant functional traits.
This finding can be related to the stronger impact of elevation on the environmental conditions (light and
GDH), compared to the impact of the microclimatic gradients (forest density and distance to forest edge).
Furthermore, the gradient in forest density might be too small to detect strong impacts on plant
performance, as even the most 'open' forests studied here were characterized by still relatively high levels

400 of canopy closure (open: 87.2 %, intermediate: 93.4 %, dense: 96.0 %; see SI Appendix 2).

401 Light availability was a key driver for many functional traits of A. nemorosa. Light had a negative impact on 402 SLA, both within the forest transect and for the control plots outside of the forest (SI Appendix 3), which 403 confirms the general finding in numerous studies that almost all taxa produce leaves of higher SLA when 404 grown in deeper shade, to maximize light interception per gram leaf (Reich et al., 2003; Poorter et al., 405 2009). This could also explain the decrease in SLA with elevation, as we found higher light levels at high 406 elevations. We found a positive effect of light availability on plant biomass, seed mass and seed number, 407 but this positive effect decreased with increasing GDH. Generally, warming increases plant photosynthetic 408 capacity (Liang et al., 2013; Kaiser et al., 2015; Zhu et al., 2020), and this might explain the observed 409 interaction between light availability and GDH on plant performance. With increased photosynthetic 410 capacity, light-use efficiency increases, and plants respond less to enhanced light availability as light is no 411 longer (or to a lesser extent) a limiting factor for plant growth. The interactive effect of light and GDH on plant performance in our study could explain why A. nemorosa is not confined to closed forests close to its 412 413 northern limit. With lower GDH, the positive effect of light availability on plant performance increased. 414 Hence, individuals could benefit more from expanding their range to open habitats with high light

415 availability at high latitudes with low levels of GDH. At higher levels of GDH, on the other hand, plant 416 performance was higher at low light availability (SI Appendix 10), which could explain why the occurrence 417 of A. nemorosa is more restricted to forest habitats in Central Europe (except for mountainous areas, but 418 here again, temperatures will be lower). The interactive effect between light and GDH suggests that, if GDH 419 increases in our study region due to climate warming, the positive effect of increased light availability on A. 420 nemorosa performance might disappear, potentially jeopardising its performance in open habitats. Our 421 analysis of A. nemorosa in the control plots outside of the forest (see SI Appendix 3) also suggests that light 422 is a key driver for the species in the study region, as we found a clear positive impact of light availability on 423 germination rate.

Interestingly, other than its interactive effect with light availability, warming was not an important driver of
plant functional traits within the forest transects in our study region. This is in contrast to earlier studies
that reported pronounced positive effects of warming on the reproductive success (seed mass and
germination) and vegetative growth (plant height and biomass) of *A. nemorosa* (Graae et al., 2009; De
Frenne et al., 2010; De Frenne, Brunet, et al., 2011).

429 In addition to the elevational gradient, the competition removal treatment also had an impact on several 430 functional traits. Plant biomass and seed mass decreased when the neighbouring vegetation (mainly 431 bryophytes) was removed, and in open forests, this was also the case for germination rate. As we did not 432 find an effect of the competition treatment on the climatic conditions (temperature and light) in the 433 subplots, it is likely that competition removal affected plant performance through its impact on 434 belowground conditions, such as soil temperatures, moisture conditions and nutrient availability (Gornall et 435 al., 2011; Soudzilovskaia et al., 2011). The removed vegetation typically consisted mainly of mosses and 436 very short shrubs, which could explain why the data loggers, installed at 10 cm above the soil surface, did 437 not detect this treatment in the microclimate (light and GDH). As we did not measure these belowground 438 conditions, it is difficult to explain the mechanisms behind our observations, but our results suggest that in 439 our study region, the facilitative effects of neighbouring vegetation (such as creating a favourable soil 440 microclimate) are more important than the competitive effects (such as competition for water or 441 nutrients).

442 Here, we focused on four environmental drivers: light availability, temperature, soil pH and soil organic 443 matter. However, several other environmental factors such as herbivory, precipitation, soil microbiology, 444 etc. could simultaneously affect plant performance, and should be taken into account in future studies to 445 obtain a more comprehensive understanding of the variation in plant functional traits across the study 446 region. Furthermore, as A. nemorosa also propagates clonally with rhizomes, it would be informative to 447 include rhizome growth characteristics to assess plant performance and colonization capacity in future 448 research. This will provide a more comprehensive understanding of the mechanisms that drive the plant's 449 response to environmental change. For instance, it is likely that rhizome growth will benefit from higher 450 winter temperatures in the northern part of the distribution range (Philipp et al., 2007), resulting in a 451 higher colonization potential. 452 In conclusion, our findings show that, close to its northern distribution range edge, A. nemorosa plant

performance benefits from higher light levels, although this benefit decreases with warming. This
interactive effect of light and temperature could explain why the species is often found outside forests at
high latitudes, but also suggests that with future climate warming, the species' performance might

456 decrease outside forests.

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