

Khumanand Sharma Dhungana

Microclimate Effects on Functional Traits of *Anemone nemorosa* at an Elevation Gradient in Norway

Master's thesis in Biology (Ecology, Behaviour, Evolution and Biosystematics)

Supervisor: Prof. Bente J. Graae, Prof. Pieter De Frenne, Co-Supervisor: Dill Prasad Rijal

November 2019





Anemone nemorosa

Abstract

Microclimatic gradients have a large influence on population growth rates of species and limit their distributions. Assessing changes in plant functional traits along microclimatic gradients is useful for understanding the drivers of climate responses in species and the mechanisms related to such responses. For instance, changes along climatic gradients may relate to temperature, moisture, or to associated drivers such as light or competition, and they may be caused by limitations in growth, reproduction or survival, which again reflected in changes in the functional traits. To understand, how microclimate along elevation and forest gradient affect plant functional traits, we selected a slow colonizing forest herb *Anemone nemorosa* as a model plant. This thesis combines observational and semi-experimental approaches to answer the following questions: (1) How is growing degree hours and light can be affected along the elevation and forest gradient as well as competition treatment? (2) Does competition along elevation and forest gradient have an effect on the performance of *A. nemorosa*? (3) Do microclimatic factors (light and temperature) have an effect on the functional traits of *A. nemorosa* along different elevations. We measured plant functional traits of *A. nemorosa* and microclimate and soil variables from three different elevations and along the forest gradients in deciduous forests in Central Norway. We used linear mixed effect model to find the answers to all our research questions.

We found that GDH and mean light intensity decreased towards the interior of the forest. We observed higher GDH in plots without other competitors compared to those that had other competitors within the plots. Plant height and leaf dry matter content increased at higher elevation and towards forest interior and when competitors were removed, while plant biomass and specific leaf area decreased at higher along elevation and towards forest interior and when competitors had been removed. GDH negatively affected plant height and LDMC but positively affected SLA and plant biomass. Surprisingly, the light did affect to functional traits of *A. nemorosa*. We conclude that taller *Anemone* with lower SLA, higher LDMC, and lower plant biomass was present at a higher elevation performed better and seems to adapted in higher elevation. Likewise, increased *Anemone* height at forest interior indicated positive adaptive strategies under canopy. Likewise, shorter *Anemone* with higher specific leaf area, in competition plots, indicated weak competitors' forest outside, at forest edge and near to forest edge.

Key words: *Anemone nemerosa*, functional trait, competition treatment, plant performance.

Acknowledgements

This research work is a part of Forest microclimate assessment project (FORMICA) and it was the great opportunity to work and understand the goal addressed by of FORMICA in climate change era. I would like to express my special appreciation and thanks to my supervisor Professor Bente J Graae, for your outstanding supervision throughout the research. From the beginning of this research, your motivation, enthusiasm, the way of guiding, your encouragement, tremendous mentor, shaped, and sharpened me in the research field. I would like to express my special thanks to my supervisor Professor Pieter De Frenne and project leader of FORMICA who continuously suggested and solved the statistical confusion and problems during data analysis. I would like to thank my co-supervisor Dilli Prasad Rijal for his guidelines in this work. I am thankful to Sanne Govaert (PhD scholar, Ghent University, Belgium and my friend Bishnu Timilsina for thoroughly reading the thesis manuscript and providing your valuable feedback. I would like to say special thanks to Raju Rimal for sharing your knowledge in R.

I am really thankful to Sigrid Lindmo (Senior Engineer, Department of Biology), Huri my colleague, and Audrey (from France) for your help during field visits many times, beginning from establishing experimental plots, until the data collection.

Last but not least, my huge thanks to my beloved wife Tara Dungana and to both of my sons Binit Dhungana and Sumit Dhungana for supporting me throughout this study.

“This thesis is dedicated to my late parents, for their immense effort to shape my life”

Table of content

Contents

Abstract	3
Acknowledgements	4
List of Figures	6
List of Tables	7
Abbreviation	7
1. Introduction	8
1.1 Research Question	12
1.2 Hypothesis	12
2. Materials and Methods	13
2.1 Study species: <i>Anemone nemorosa</i> (L.)	13
2.2 Study Area:	13
2.3 Study Design	15
2.4 Soil analysis	16
2.5 Environmental Variables	18
2.6 Growing degree hour and mean light	18
2.7 Statistical Analysis	19
3 Results	20
3.1 Effect of competition treatment and plot distance on growing degree hour (GDH) and light	20
3.2 Effect of altitude, plot distance and competition treatments on traits of <i>A. nemorosa</i>	21
3.3 Effect of light and GDH on functional traits of <i>A. nemorosa</i>	24
4. Discussion	28
4.1 Effects of plot position, altitude and competition treatment on GDH and light	28
4.2 Effect of elevation gradient on <i>A. nemorosa</i> trait	28
4.3 Effect of forest gradients on <i>A. nemorosa</i> traits	30
4.4 Effect of competition on <i>A. nemorosa</i> traits	30
4.5 Effect of GDH and light on <i>A. nemorosa</i> traits	31
5. Conclusion:	32
Bibliography	33

List of Figures

Figure 1 The sampling sites (Low altitude, Mid altitude, Higher altitude).	13
Figure 2 Layout of field study design (9 sites with 6 experimental plots in each sites)	15
Figure 3 A sampling plot of 20x40 cm ² , divided into two halves (20x20 cm ²), The center is marked with a metal stand	16
Figure 4 Oven drying of soil at 550 ° C for 6 hours	17
Figure 5 Preparation of soil samples to measure pH	17
Figure 6 Data loggers were placed in sampling plots sensors facing upward direction	18
Figure 7 Growing degree hours (GDH) are negatively negatively affected by (a) plot distance (r= -0.37) and (b) soil pH (r= -0.33)	21
Figure 8 The difference in mean light in plot distance and plots with and without competition	21
Figure 9 a,b,c,d: The variation of LDMC (top left), plant biomass (top right), (bottom) and SLA (bottom right) in response to competition.	25
Figure 11 a, b, c, d: The variation of LDMC (top left), plant biomass (top right) plant height, (bottom left) and SLA (bottom right) in response to Altitude and competition.	26
Figure 12 a, b, c, d: The effect of GDH on LDMC (top left), plant biomass (top right) , plant height, (bottom left) and SLA (bottom right).	26
Figure 13 Availability of organic matter in different elevation.	27

List of Tables

Table 1 Locations, latitude, longitude, altitude and major vegetation of the research sites	14
Table 2 Results from linear mixed-effects models testing the effect of altitude, plot distance and competition on growing degree hours and mean light.	20
Table 3 Results from linear mixed-effects models testing the effect of altitude, plot distance and competition on different functional traits of <i>Anemone nemorosa</i> .	22
Table 4 Results from linear mixed-effects models testing the effect of GDH and light on different traits of <i>Anemone nemorosa</i> .	24

Abbreviation

PFT	Plant functional Trait
GDH	Growing degree hours
SLA	Specific leaf area
LDMC	Leaf dry matter content

1. Introduction

Climate change is considered one of the major threats to biological diversity. The global annual temperature has increased by approximately 0.85°C over the past century. The temperature is projected to increase by 2 to 4 °C until the 21st century (Stocker et al., 2013). The rapid increase in temperature due to elevated level of CO₂ affects the environment, and influence species distribution and community composition (Gaston, 2003), and vital population phenomena in terms of individual's growth, survival, births, deaths, immigration and, emigration (Ehrlén and Morris, 2015).

The distribution of plant communities is affected by both regional climatic conditions and local microclimatic conditions. The microclimate experienced by the living organisms of a particular place is light, temperature, soil moisture, radiation, and wind. (Bramer et al., 2018). Plant exposure to microclimatic variation differ from temporally and spatially. For example, at the daytime, leaves, and stem can experience higher temperature than the temperature of the air surrounding these tissues, especially when wind speed is not high (Bramer et al., 2018; Chen et al., 1999; De Frenne et al., 2013a). Forests play a crucial role in facilitating the microclimate experienced by understory plants by lowering air and soil temperatures, irradiance, and evapotranspiration (De Frenne et al., 2010). Therefore, “increases in forest cover over large spatial and temporal scales can slow the ¹thermophilization of understory plant communities during periods of climate warming” (*sensu* De Frenne et al., 2013a; Lemke et al., 2015).

Forest gradient or plot distance or plot position will be interchangeable words used throughout this research study, which means the distance from the outer edge of the forest to forest interior. The study of forest gradient encompasses a highly variable environment. Along the forest gradient, the presence of small or large canopy openings greatly affects the features of the understory microenvironment (Antos, 2002; De Frenne et al., 2013b). Because different tree species (overstory) intercept variable amounts of light intensity and creates an understory layer with much higher resource levels such as light and temperature (Antos, 2002). Consequently, forest understory plants tend to form a distinct group of species associated with that type of habitat (Antos, 2002). These forest species are probably poor competitors in more open habitats and normally demonstrate features typical of stress-tolerant species, such as

¹ According to (Stevens et al., 2015) “Climate change is likely to shift plant communities towards species from warmer regions, a process termed ‘thermophilization’”.

naturally slow growth rates, and conservative patterns of resource allocation (Antos, 2002). Here the focused species, *Anemone nemorosa*, is commonly distributed throughout the temperate deciduous forest in my all studied sites and exhibits shade tolerance adaptation to low light condition (De Frenne et al., 2012).

Light is a key factor that is essential for regeneration, growth, and development (Valladares and Niinemets, 2008). Plants can respond by changing their physiological and morphological traits in response to change in light. For instance, under shading, most plant species in open habitats elongate their stem and petioles, bend their leaves upwards, or reduce branching to attain light (Givnish, 1988; Gommers et al., 2013; Griffith and Sultan, 2005). Conversely, if plants grow faster than surrounding plants then these plants adopt as shade-tolerant (e.g. some herbaceous plants from forest understories), and increase their specific leaf area (SLA), to optimize carbon gain (Givnish, 1988; Gommers et al., 2013; Griffith and Sultan, 2005).

Temperature is one of the crucial factors which affects plant physiology and determines plant performance. It also strongly influences the geographical and altitudinal distribution of plants (Walther et al., 2005). Therefore, change in distribution of the plant species indicates change in the temperature pattern among others of certain geographical landscape. For instance, Parmesan and Yohe, (2003) reported increased numbers of species along an elevation gradient over the time which indicates an upward shift of species. Study of plant distribution, their functional traits, and growth along an elevational gradients gives clear picture of the impact of microclimate (Parmesan and Yohe, 2003). Because lower and upper elevation are distinguished by their biotic and abiotic characteristics. Lower elevation most often has higher temperature, different atmospheric humidity while high altitude is characterised by lower temperature, high solar radiation, quick change in temperature, and low partial pressure of air. Changes in any one of these factors (e.g. temperature, solar radiation, atmospheric pressure, and transpiration rate) affect plant performance which reflected in values of plant functional traits (PFT). Yüksek et al., (2013) stated that plant height, SLA, plant biomass, leaf dry matter content, plant structure, and physiology have been detected to change along elevational gradients. In addition to varying with elevation, climate significantly affects soil properties such as organic matter, soil pH, soil moisture, nitrogen, phosphorous changing with chemical, physical, and biological processes.

Plant functional traits (PFT) are any morphological, physiological, or phenological structures that can be measured at individual level without interaction with environment or any other organizational level (*sensu* Violle et al., 2007). According to Reich et al., (2003) and Violle et

al., (2007) functional traits are defined as morphological, physiological and phenological properties which indirectly influences on fitness of individuals by its effects on the performance in terms of growth, survival, and reproduction of plants. In my study PFT are used to study of single species *A. nemorosa* in responses to environmental variation. Broadly, PFT are used to examine the possible changes in their structure in response to climate change or the mode of land use (Poorter, 2007; Poorter et al., 2018). Therefore, study of PFT can answer how plant traits are related with environmental factors and where a species can grow and establish successfully. For instance, plant's performance depends on its height/size and leaf traits. So, these traits can be used to find the variation in plant function and diversity.

SLA is one of the most useful and informative functional traits and is calculated as the one-sided leaf surface area per unit dry weight. It is an indicator of eco-physiological characteristics such as relative growth rate, stress tolerance, and leaf longevity (Lavorel and Garnier, 2002). A higher SLA means reduced leaf density, or reduced leaf thickness or combination of both. It shows a trade-off between acquisition of resource and their conservation, which has a strong correlation with leaf life span, growth rate, and competitive ability (Wright et al., 2004). SLA strongly correlates with light, temperature and resources availability which is heterogeneous along elevational gradients. SLA generally decreases with increasing altitude, which may indicate responses of plants to decreased temperature, increased light, or changes in resources availability (Poorter et al., 2009; J F Scheepens et al., 2010). Leaf Dry Matter Content (LDMC), the ratio of leaf dry mass to fresh mass, is also associated with growth and survival of plants. According to Poorter and De Jong, (1999) both SLA and LDMC, as mentioned earlier, they are highly correlated. Both these traits are involved in trade-offs between rapid biomass production (high SLA, low LDMC species) and efficient conservation of nutrients (low SLA, high LDMC species). Since SLA and LDMC are good indicator traits of resource-use strategies, these can be utilized to study the variation in resource use strategy of plant species along the environmental gradients. (Westoby, 1998)

Plant height is also a central functional trait because it is associated with competition for light and helps explaining how a species lives, grows, and reproduces. There is a strong association between plant height and other plant traits such as biomass and leaf dry matter content (LDMC), leaf area ratio, and leaf nitrogen per area (Falster and Westoby, 2003). Plant height decrease with increasing altitude since it allows the plants to reduce reproductive investment Moles et al., (2009). Additionally, plants are shorter at higher elevation due to high radiation,

low-temperature, high wind, and aridity. It is caused due to lower competition for light and poorer soil condition. Furthermore, another reason for decreasing plant height with increasing altitude is reduction of carbon gain (Moser et al., 2007).

Competition is an important ecological phenomenon regulating plant communities, along resources availability (Navas and Violle, 2009). The effects of competition is widespread and can investigate between coexisting species by studying their performance via their functional traits. Light is a limiting-factors for forest understory herbs and shade-tolerant forest herb species are less sensitive to shading by their neighbours (Zhang et al., 2016). Because shade tolerance species can allocate their maximum resource resources to defence in compare to growth (Zhang et al., 2016). Therefore, they are less vulnerable to than light-demanding species in defensive terms. Studies has shown that shade-tolerant species are likely to maximize their survival and growth via elongation of height, branches and leaves which help to capture maximum light resources (Liu et al., 2016). This study will reveal the effect of competition with other species in *Anemone*'s functional traits along forest gradient, and elevation gradient where the resources (light and temperature) is a limiting factor.

Forest herbs are especially at risk of climate change for several reason (De Frenne et al., 2012, 2011a). For instance, firstly many forest herbs have different biological traits which can limit migrating (dispersal ability) in response to changing climate. Because different traits respond differently in n response to changing climate. Secondly, due to fragmentation and partial connectivity of forest areas and due to different human activity in agriculture, roads, and development may increase the threats from natural climate change. Furthermore, the geographical/environmental distributions of a few forest herbs may still be affected by past climate change by which they quickly response to modern climate change (Bellemare and Moeller, 2015). However, many forest herbs are not able to quickly trace suitable environmental conditions, therefore, their existance may mainly depend on the conservation of existing forest habitats within their real distribution range (De Frenne et al., 2011a; Honnay et al., 2002; Vanneste et al., 2017).

This study is focused on *A. nemorosa*, which is an understory forest herbs particularly vulnerable to climate change due to dispersal limitation (De Frenne et al., 2011a). *A. nemorosa* flowers and photosynthesize at different periods in different parts of Europe, where flowering depends largely on light, day length, and temperature (Shirreffs, 1985). In Northern parts of Europe, day length is greater in the summer, and the canopy may be more open than in the Southern. In the Southern part of Europe, they are mostly limited to the cooler patches.

In the Southern part, *A. nemorosa* starts flowering from March while in the Northern part, it flowers from May to June (Shirreffs, 1985). More specifically, in Norway, this species behaves differently than in other parts of Europe. In Norway, this species appears in first week of May; flowers in beginning of June and seed matured at the end of the June. That is because, in Norway, day length are longer, so this species receives higher light and lower temperature as the day length increase in summer. We expect that due to availability of longer days, longer light availability and lower temperature availability *A. nemorosa* get higher opportunity to grow bigger, perform better and spread at higher elevations.

1.1 Research Question

1. Do elevation, forest gradients and competition treatment have an effect on the microenvironment (light and GDH)?
2. Does competition along elevation and forest gradients have an effect on the performance of *A. nemorosa*?
3. Do microclimatic factors (light and GDH) have an effect on the functional traits of *A. nemorosa* along different elevational gradient?

1.2 Hypothesis

We hypothesized that ²plant performance depends on temperature and light availability. Higher plant performance in the northern region and higher elevation may be due to lower temperature and lower but longer light availability or due to both factors. This is due to different day lengths but also due to light availability caused by canopy cover. The competition with other co-occurring species can be expected to be decreased performance of *A. nemerosa* by their functional traits.

²Plants (*A.nemorosa*) with taller height, low SLA and high LDMC at higher elevation are supposed to be performed better. Likewise, taller plant with high SLA with low LDMC are the favourable traits supposed to perform better at forest interior (Pickering and Venn, 2013).

2. Materials and Methods

2.1 Study species: *Anemone nemorosa* (L.)

Anemone nemorosa (Ranunculaceae) is common in European forest, from Northern Spain to Northern Sweden (67 °N) and from Ireland to Western Russia in the understory of ancient deciduous forest. (De Frenne et al., 2010 and De Frenne et al., 2011). *A. nemorosa* is a perennial, spring flowering hemicryptophytic herbaceous plant, typically growing on nutrient rich mildly acidic soil. This species is also found growing in open areas. Shoots appears between March and May, and flowering starts a few weeks after appearance of the shoots. The white hermaphroditic flowers of *A. nemorosa* are often insect pollinated and occasionally self-pollinated. The species produces 10-30 achenes per flower, and propagates widely clonally with rhizomes (De Frenne et al., 2010).

2.2 Study Area:

This research was conducted in three different locations representing three different altitudinal belts: at the lowest altitude close to Trondheim Fjord, at mid-altitudinal belt close to Oppdal, and at the highest altitude in the Dovre mountains. Within each of these three altitudinal belts, three different sites were selected as represented in Fig 1. and Table 1.

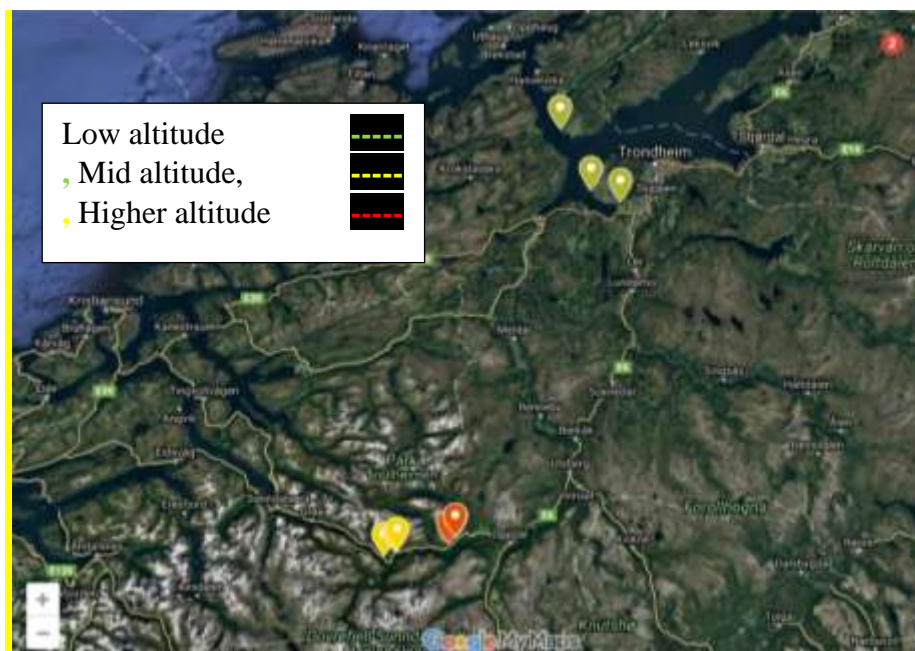


FIGURE 1 THE SAMPLING SITES (LOW ALTITUDE, MID ALTITUDE, HIGHER ALTITUDE).

TABLE 1 LOCATIONS, LATITUDE, LONGITUDE, ALTITUDE AND MAJOR VEGETATION OF THE RESEARCH SITES

Location	Site No	Research site	Latitude	Longitude	Altitude (m)	Major Vegetation
Lowest altitude – Trondheim fjord	1	Vedvik	62.33865	009.04.734	23	<i>Alnus glutinosa, Sorbus aucuparia, Anemone nemorosa and Oxalis acetosella</i>
	2	Lauglo	62.33865	009.04.734	43	<i>Alnus glutinosa, Sorbus aucuparia, Anemone nemorosa and Oxalis acetosella</i>
	3	Brørskiftberga	62.33865	009.04.734	45	<i>Alnus glutinosa, Sorbus aucuparia, Anemone nemorosa and Oxalis acetosella</i>
Mid-altitude – Oppdal	4	Krokbygda	62.34.485	009.08.672	223	<i>with Alnus glutinosa, Sorbus aucuparia, Anemone nemorosa and Oxalis acetosella</i>
	5	Vollan	62.34.293	009.07.960	269	<i>Betula pubescens, Alnus glutinosa and Pinus Sylvestris, Anemone nemorosa and Oxalis acetosella</i>
	6	Gravem	62° 33' 49.23"	9° 4' 38.0604"	269	<i>Betula pubescens, Alnus glutinosa and Pinus Sylvestris, Anemone nemorosa and Oxalis acetosella</i>
Highest altitude – Drove	7	Furans	62.36.271	009.25.629	660	<i>Betula pubescens, Pinus sylvestris, , Sorbus aucuparia, Anemone nemorosa and Oxalis acetosella</i>
	8	Furans	62.36.271	009.25.629	660	<i>Betula pubescens, Pinus sylvestris, , Sorbus aucuparia, Anemone nemorosa and Oxalis acetosella</i>
	9	Kvammen	62.35.380	009.23.583	663	<i>Betula pubescens, Pinus sylvestris, , Sorbus aucuparia, Anemone nemorosa and Oxalis acetosella</i>

2.3 Study Design

All the field work for this research were performed between May and June 2018. At each site, a transect was established starting from outside of a forest to 100 meters into the forest. Along this transects, six 20x40 cm² plots were placed so that first plot was established outside of the forest and the rest of the five plots were inside the forest in 2m, 5m, 13m, 50m, 100m from the forest edge. In each plot at least 5 *Anemone* were presents.

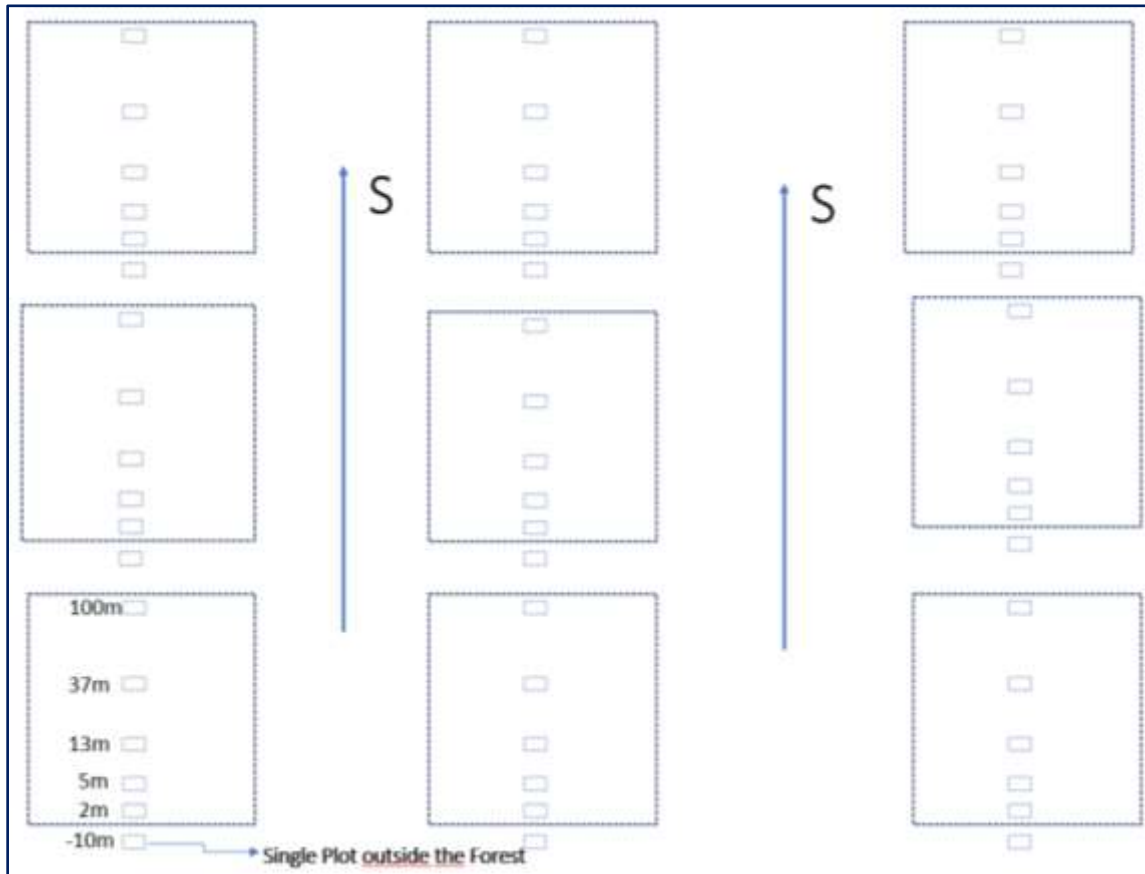


FIGURE 2 LAYOUT OF FIELD STUDY DESIGN (9 SITES WITH 6 EXPERIMENTAL PLOTS IN EACH SITES) Each experimental plot of 20x40 cm² was divided into two half 20x20 cm² each. In half of the plot, all plants, other than *A. nemorosa*, were removed, whereas the other half was kept intact giving opportunity to evaluate the effect of competition from other plant species to the performance of the *A. nemorosa* plants. We call each ramet of *A. nemorosa* a plant in this study, though we are aware that they are basically ramets. In each plot the plant height, leaf area leaf dry matter content, specific leaf area, and plant biomass were measured for at least 5 individuals of *A. nemorosa*. In addition, the environmental variables, elevation, temperature and light availability were recorded.



FIGURE 3 A SAMPLING PLOT OF 20X40 CM², DIVIDED INTO TWO HALVES (20X20 CM²), THE CENTER IS MARKED WITH A METAL STAND

In order to measure the plant height, leaf area, specific leaf area, and plant biomass, five individual plants from each of the competition and non-competition plots were harvested at the base of the stem. These fresh plants were kept in a plastic bag with wet paper in order to avoid desiccation and brought to the laboratory immediately after harvesting. In the lab, plant height, leaf fresh weight, and leaf area were measured within 72 hours. Height of the harvested plant was measured (in cm.) from the base of the stem to the highest photosynthetic expanded tissues. From each plant, one healthy leaf was separated from the plant, and leaf area was scanned by a canon digital scanner. At the same time, fresh weight of leaf was measured on a balance. The plant and leaf were put together in a paper bag and dried for 72 hours at 60°C and reweighed separately to calculate dry leaf mass and plant biomass. The SLA was obtained by dividing the fresh one-sided leaf area by its oven dried mass. Similarly, leaf dry matter content (LDMC), the ratio of the leaf dry mass to fresh mass was calculated by dividing fresh weight of leaf (Cornelissen et al., 2003).

2.4 Soil analysis

From each of the 54 plots, soil samples were collected from 0-15 cm depth. All soil samples were dried in oven for 72 hours at 60 °C, finely ground and sieved with 2mm sieves.



FIGURE 4 OVEN DRYING OF SOIL AT 550 ° C FOR 6 HOURS

Soil pH was measured by dissolving 10 g fine ground soil into 0.001 M CaCl₂ · 2H₂O and then measuring in the supernatant after 30 minutes of settling (McBratney et al., 2000).

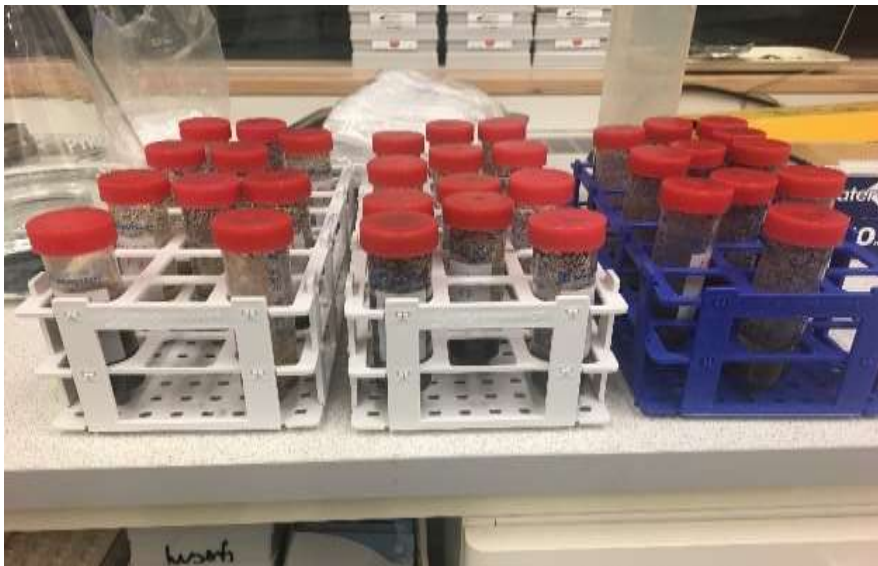


FIGURE 5 PREPARATION OF SOIL SAMPLES TO MEASURE PH

Total soil organic matter (SOM) was measured as loss on ignition at 550 °C for 6 hours after (Konare et al., 2010).

$$\text{LOI (\%)} = (\text{Weight initial T} - \text{Weight ignition T}) / \text{Weight initial T} \times 100.$$

2.5 Environmental Variables

Temperature and mean light intensity were calculated from 22.May 12.00 to 13 June 17.00 pm when the plants were collected. Data for temperature and light intensity (lux) were recorded using waterproof pendent Temperature/Light 64K Data loggers (UA-002-64) (Onset Computer Corporation, MA; USA). 108 data loggers (one for each competition and non-competition subplots) were placed in the middle of each plot. The sensors of the data loggers were oriented upward to capture natural light.



FIGURE 6 DATA LOGGERS WERE PLACED IN SAMPLING PLOTS SENSORS FACING UPWARD DIRECTION

2.6 Growing degree hour and mean light

Growing degree hours (GDH) is proposed as the standardized protocol, providing adequate accuracy and high precision used. Here, Growing Degree Hour (GDH) was used to test the effect of total accumulated temperature on the performance on plant height, plant biomass, SLA and LDMC. GDH is calculated on the basis of hourly recorded temperature on the Hobo data temperature-light loggers (Körner and Hiltbrunner, 2018). GDH was calculated with 5 °C as base temperature considered to be minimum temperature required for the sprouting of *Anemone* (De Frenne et al., 2010). Mean light was calculated from the recording in the data loggers every hour during the field experimental period.

2.7 Statistical Analysis

We performed Linear mixed effect model with lmer () function. Because Linear mixed effect models include, a mixture of both fixed and random effects. (Alain F. Zuur Elena N. Ieno Graham M. Smith, 2007; Kuznetsova et al., 2017). The statistical analysis was carried out using a three steps approach. All three steps were performed with linear mixed effect model (LME), with two-way interactions. Two-way interaction performed to see the interaction effect on response variables with each other. In my all models, sites and plots were taken random effect, where plots are nested within the sites. The variables I used in different models, were in different scale and varied differently. Therefore it was necessary to scale them before modelling, so that a single variable do not influence the model more than others due to their large value (Alain F. Zuur Elena N. Ieno Graham M. Smith, 2007).

In the first approach, we tested the effects of altitude, competition treatment and plot distance on GDH and mean light intensity. Here I used two separate models for GDH and mean light. In this approach GDH and mean light were treated as response variables while altitude, competition treatment, and plot distance and were used as predictor variables. Within this step, soil pH and organic matter were taken as covariates (However, these soil pH and organic matter are not my primarily interesting factors, but I want to see the effect of their presence).

In the second step, we tested the effects of geographical variation (altitude, plot position, and competition treatment), on the performance of *A. nemorosa*. Where plant height, plant biomass, specific leaf area, and leaf dry matter content considered as the response variable, and soil pH and soil organic matter were taken as covariates. Four different models, for each response variables, were developed to test the effect of fixed and random variables.

In the third step the competition treatment, altitude, and plot distance were excluded to see how the light and GDH affect plant height, plant biomass, specific leaf area, and leaf dry matter content. In this model mean-light and growing degree hours (GDH) are fitted in the same model as they are not correlated with each other. In this model four different models for each response variables, were approached to see the effect of competition treatment, altitude and plot distance and interaction between them. Also in this model soil pH and soil organic matter were considered as covariates. All the analyses and data visualization were performed using R version 3.5.3 (R Core Team, 2019). Further, to get the data visualization between response and predictor we used ggplot2, and effect plots.

3 Results

3.1 Effect of competition treatment and plot distance on growing degree hour (GDH) and light

Growing degree hours were negatively affected by plot distance from the forest edge, and soil pH, (Table 2, figure 7a and 7b). Results show that the mean light intensity is significantly different between competition and non-competition plots (Table 2). The mean value of light is higher in non-competition (Figure 8).

Table 2 Results from linear mixed-effects models testing the effect of altitude, plot distance and competition on growing degree hours and mean light.

Response	Parameters	Estimate	Df	t-value	Pr(> t)
GDH	Intercept	8134.25±954.10	200.97	8.53	p<0.001
	Plot distance	-354.60±88.52	43.47	-4.006	p<0.001
	Altitude	-117.69±206.15	7.91	-0.57	0.583
	Competition VS non-Competition	63.49±27.13	447.93	2.34	p<0.05
	Soil pH	-740.17±216.13	220.59	-3.42	p<0.001
	Organic matter	-73.48±56.55	102.08	-1.29	0.197
	Plot distance: altitude	26.20±91.884	42.36	0.285	0.776
	Plot distance : Competition Vs non-Competition	-16.82±27.63	448.84	-0.615	0.484
	Altitude: Competition Vs non-Competition	88.22±26.92	447.18	3.27	P<0.01
Mean Light	Intercept	44746.6±9815	127.1	4.56	p<0.001
	Plot distance	-2101.62±859.65	51.61	-2.44	P<0.05
	Altitude	-502.19±1062.27	65.74	-0.47	0.637
	Competition vs Non-Competition	1986.31±339.85	448.66	5.845	p<0.001
	Soil pH	-8361.20±2269.43	132.18	-3.684	p<0.001
	Organic matter	-766.31±485.10	66.60	-1.58	0.118
	Plot distance: Altitude	351.52±877.88	47.63	0.400	0.690
	Plot distance: Competition Vs non-competition	-1616.74±342.56	449.18	-4.72	p<0.001
	Altitude : Competition Vs non-competition	-1135.18±337.19	447.17	-3.367	p<0.001

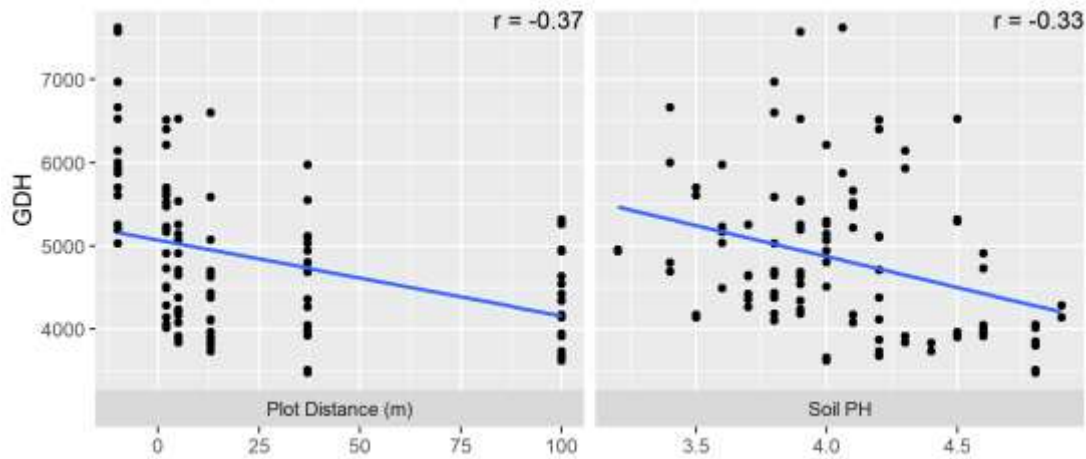


FIGURE 7 GROWING DEGREE HOURS (GDH) ARE NEGATIVELY NEGATIVELY AFFECTED BY (A) PLOT DISTANCE ($R= -0.37$) AND (B) SOIL PH ($R= -0.33$)

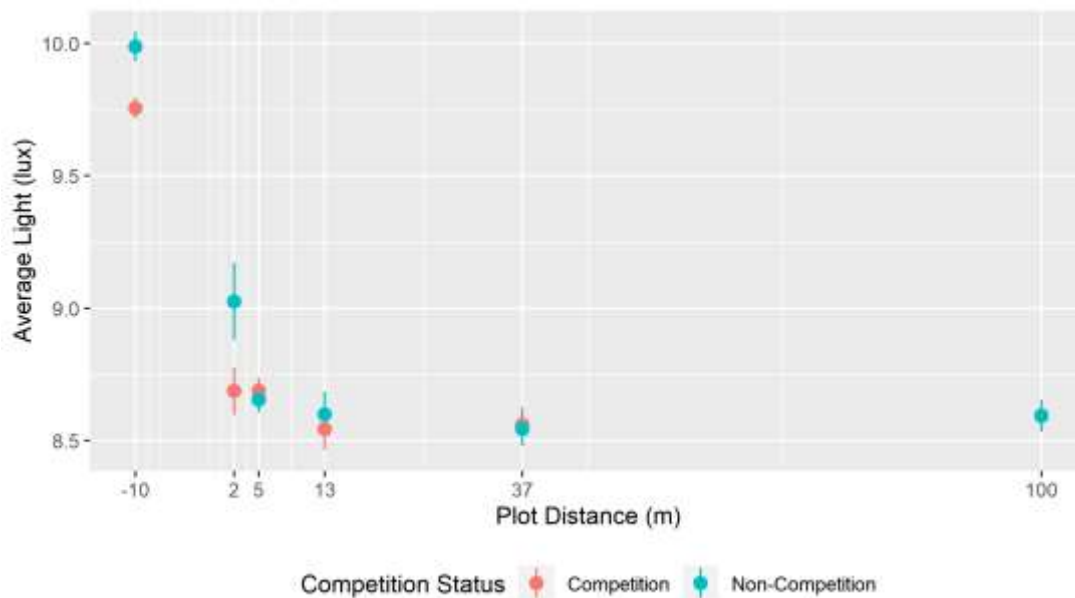


FIGURE 8 THE DIFFERENCE IN MEAN LIGHT IN PLOT DISTANCE AND PLOTS WITH AND WITHOUT COMPETITION

3.2 Effect of altitude, plot distance and competition treatments on traits of *A. nemorosa*

Plant height was positively affected by distance to edge, altitude, and competition treatment (Table 3, Figure 9,10,and 11). There was further a positive interaction between altitude and competition. Plant biomass was negatively affected by plot distance, altitude, and organic matter (Table 3). Likewise, plot distance, altitude and competition, and the interaction of altitude and competition negatively affected SLA and positively affected leaf dry matter content (LDMC) (Table 3). Average

plant height and LDMC were greater in non-competition subplots, whereas SLA and plant biomass were higher in competition plots (Figure 10 a,b,c,d).

TABLE 3 RESULTS FROM LINEAR MIXED-EFFECTS MODELS TESTING THE EFFECT OF ALTITUDE, PLOT DISTANCE AND COMPETITION ON DIFFERENT FUNCTIONAL TRAITS OF *ANEMONE NEMOROSA*.

Response	Parameters	Estimate± SE	Df	t-value	Pr (> t)
Plant height	Intercept	10.764± 0.598	23.57	17.98	p<0.001
	Plot distance	0.167±0.029	50.10	5.71	p<0.001
	Altitude	0.230±0.437	7.05	5.10	p<0.01
	Competition vs non-competition	0.692±0.026	445.49	26.40	p<0.001
	Soil pH	0.013±0.098	46.29	0.14	0.888
	Organic matter	-0.021±0.021	37.13	-0.99	0.327
	Plot distance: Altitude	0.026±0.027	32.97	0.95	0.344
	Plot distance: Competition Vs non-Competition	0.035±0.026	448.13	1.33	0.182
	Altitude: Competition Vs non -Competition	0.109±0.026	440.71	4.19	p<0.001
	Plant Biomass	Intercept	0.1015±0.0093	15.99	10.90
Plot distance		-0.001.535±0.000397	71.4	-3.85	p<0.001
Altitude		-0.03606±0.00765	6.97	-4.71	p<0.01
Competition Vs non-Competition		-0.00920±0.000432	453.056	-21.32	p<0.001
Soil pH		-0.00197 ±0.00130	47.88	-1.506	0.138
Organic matter		-0.0002714±0.000283	40.71	0.957	0.344
Plot distance: altitude		-0.0000542±30.000577	37.66	-0.15	0.880
Plot distance: competition Vs non-Competition		-0.001301±0.0004347	457.01	-2.99	p<0.01

	Altitude: Competition vs Non-competition	0.0001897±0.0004300	447.83	0.441	0.659
SLA	Intercept	27.4502±1.145	21.66	23.97	p<0.001
	Plot distance	-0.331±0.054	66.62	-6.023	p<0.001
	Altitude	-4.517±0.862	7.05	-5.239	p<0.01
	Competition vs non-Competition	-1.248±0.056	452.69	-22.282	p<0.001
	Soil pH	-0.178±0.182	49.95	0.976	0.333
	Organic matter	-0.010±0.039	41.85	-0.258	0.797
	Plot distance: altitude	-0.066±0.050	38.261	-1.325	0.193
	Plot distance: competition Vs non-Competition	0.057±0.056	456.02	1.014	0.311
	Altitude: Competition Vs Non-Competition	-0.202±0.055	447.87	-3.634	p<0.001
LDMC	Intercept	0.1723±0.00680	13.29	25.34	p<0.001
	Plot distance	0.00146±0.000255	59.19	5.73	p<0.001
	Altitude	0.0298±0.00589	7.02	5.070	p<0.01
	Competition Vs non-competition	0.00765±0.000259	448.43	29.56	p<0.001
	Soil pH	0.000989±0.000853	44.79	1.16	0.252
	Organic matter	-0.0000522±0.000186	37.29	-0.28	0.780
	Plot distance: altitude	0.000352±0.000235	34.10	1.50	0.143
	Plot distance: competition vs non-Competition	0.00124±0.000260	452.05	4.78	p<0.001
	Altitude: Competition Vs non-Competition	0.000871±0.000257	443.13	3.38	p<0.001

3.3 Effect of light and GDH on functional traits of *A.nemorosa*

The linear mixed effect model testing the effect of GDH and light on the traits of *Anemone* showed that plant height, plant biomass, SLA and plant biomass were significantly but negatively affected by GDH (Table 4, figure, 12 a,b,c,d). While, Interaction of GDH and light had only minor but positively affected to plant height and plant biomass.

TABLE 4 RESULTS FROM LINEAR MIXED-EFFECTS MODELS TESTING THE EFFECT OF GDH AND LIGHT ON DIFFERENT TRAITS OF *ANEMONE NEMEROSA*.

Response	Parameters	Estimate± SE	SE	Df	t-value	Pr(> t)
Plant height	Intercept	11.895 ±1.052	1.052	15.38	11.30	p<0.001
	GDH	-0.192±0.049	0.049	122.62	-3.84	p<0.001
	Mean light	0.0039±0.033	0.033	305.13	0.120	0.904
	Soil pH	-0.2087±0.133	0.133	46..21	-1.56	0.124
	Organic matter	-0.024±0.027	0.027	39.29	-0.89	0.377
	GDH: mean light	0.078±0.029	0.029	143.06	2.69	p<0.01
Plant Biomass	Intercept	0.085±0.015	0.015	11.60	5.41	p<0.001
	GDH	0.0034±0.00063	0.00063	110.85	5.40	p<0.001
	Mean light	-0.000449±0.000446	0.000446	267.80	-1.01	0.264
	Soil pH	0.001172±0.00155	0.00155	42.65	0.76	0.641
	Organic matter	-0.000123±0.000320	0.000320	37.55	-0.38	0.730
	GDH: mean light	-0.001110±0.000373	0.000373	104.77	-2.98	p<0.01
SLA	Intercept	25.71±2.044	2.044	13.79	12.58	p<0.001
	GDH	0.2817±0.0926	0.0926	120.59	3.04	p<0.01
	Mean light	-0.0504±0.0628	0.0628	296.67	-0.80	0.951
	Soil pH	0.01250±0.2387	0.2387	45.96	0.52	0.741
	Organic matter	0.0129±0.0497	0.0497	39.69	0.26	0.750
	GDH: mean light	-0.0628±0.0544	0.0544	128.93	-1.15	0.729
LDMC	Intercept	0.1856±0.0129	0.0129	11.76	14.28	p<0.001
	GDH	-0.00212±0.000504	0.000504	114.52	-4.21	p<0.001
	Mean light	0.0000481±0.000343	0.000343	287.79	0.14	0.186
	Soil pH	0.001595±0.00129	0.00129	43.28	-1.23	0.297
	Organic matter	-0.000187±0.000269	0.000269	37.43	0.69	0.453
	GDH: mean light	0.000712±0.000296	0.000296	121.24	2.40	p<0.05

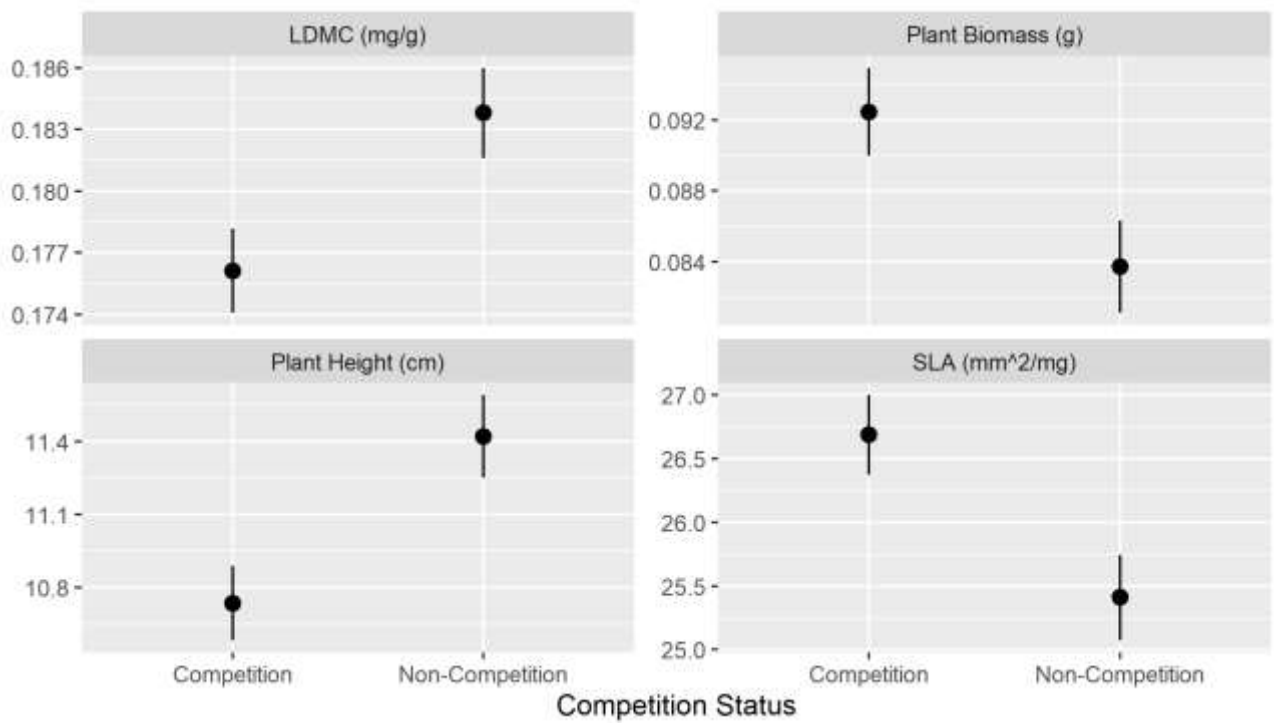


FIGURE 9 A,B,C,D: THE VARIATION OF LDMC (TOP LEFT), PLANT BIOMASS (TOP RIGHT), (BOTTOM) AND SLA (BOTTOM RIGHT) IN RESPONSE TO COMPETITION.

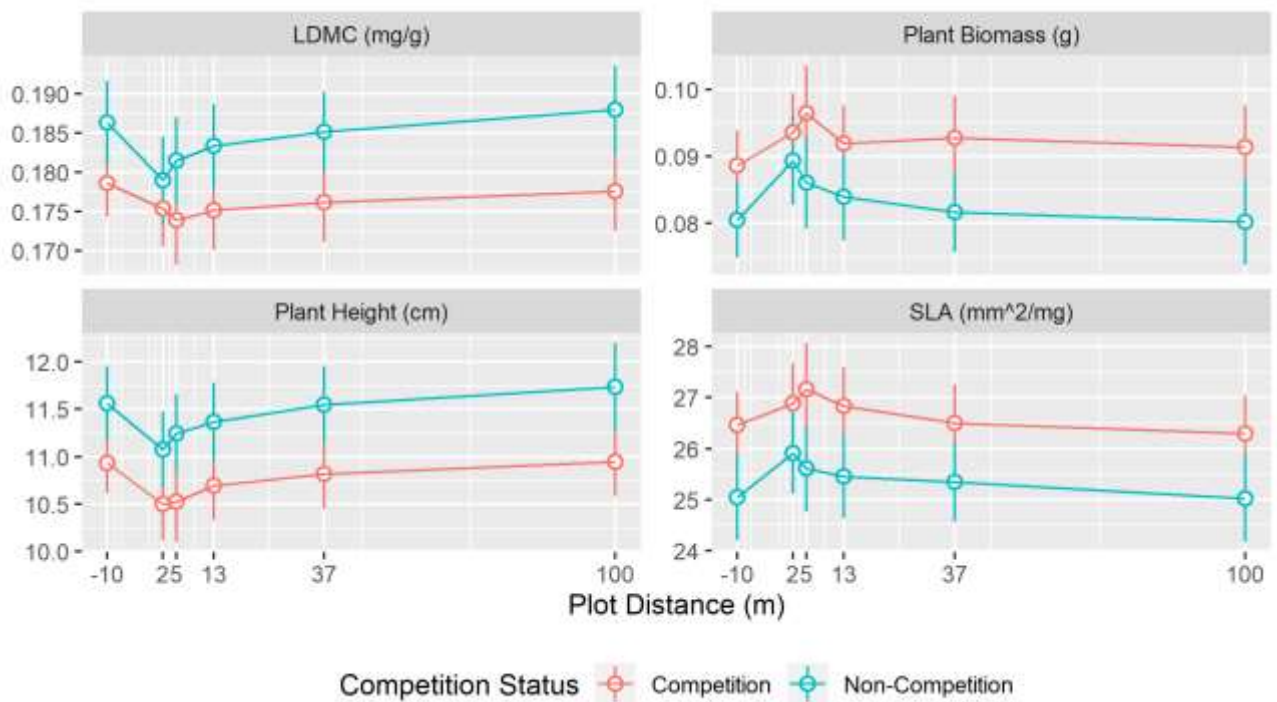


FIGURE 10 A,B,C,D: THE VARIATION OF LDMC (TOP LEFT), PLANT BIOMASS (TOP RIGHT), PLANT HEIGHT (BOTTOM LEFT) AND SLA (BOTTOM RIGHT) IN RESPONSE TO COMPETITION.

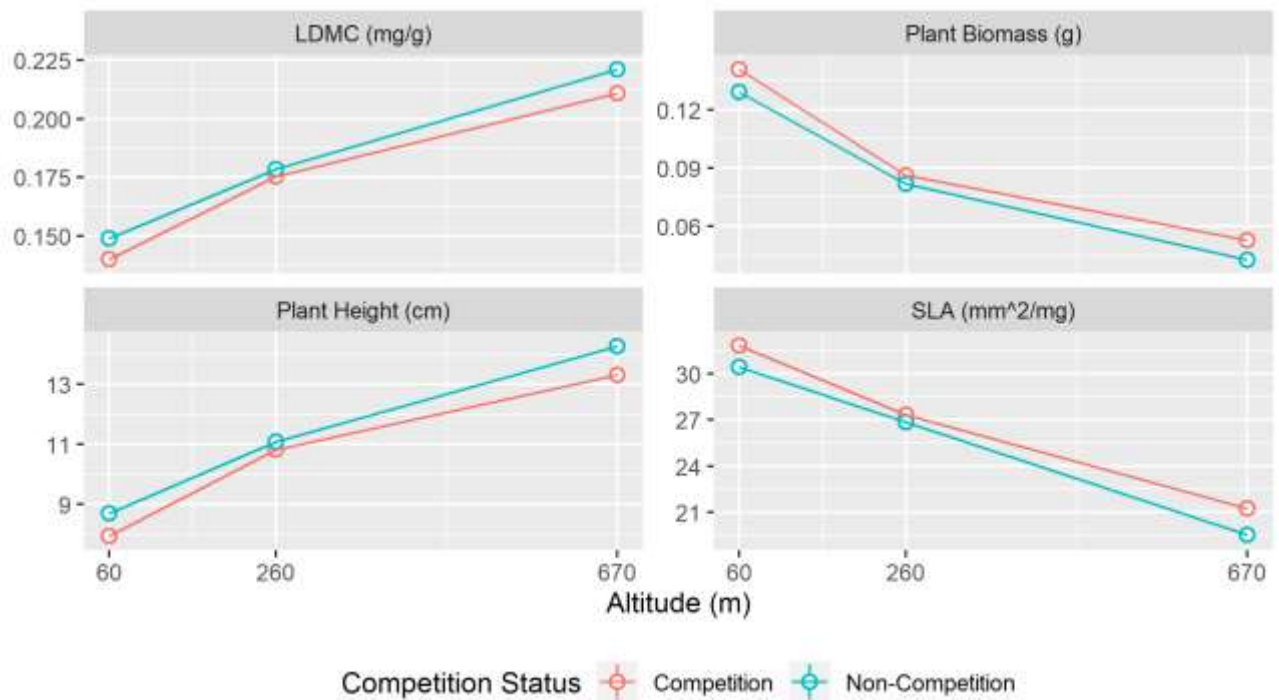


FIGURE 10 A, B, C, D: THE VARIATION OF LDMC (TOP LEFT), PLANT BIOMASS (TOP RIGHT) PLANT HEIGHT, (BOTTOM LEFT) AND SLA (BOTTOM RIGHT) IN RESPONSE TO ALTITUDE AND COMPETITION.

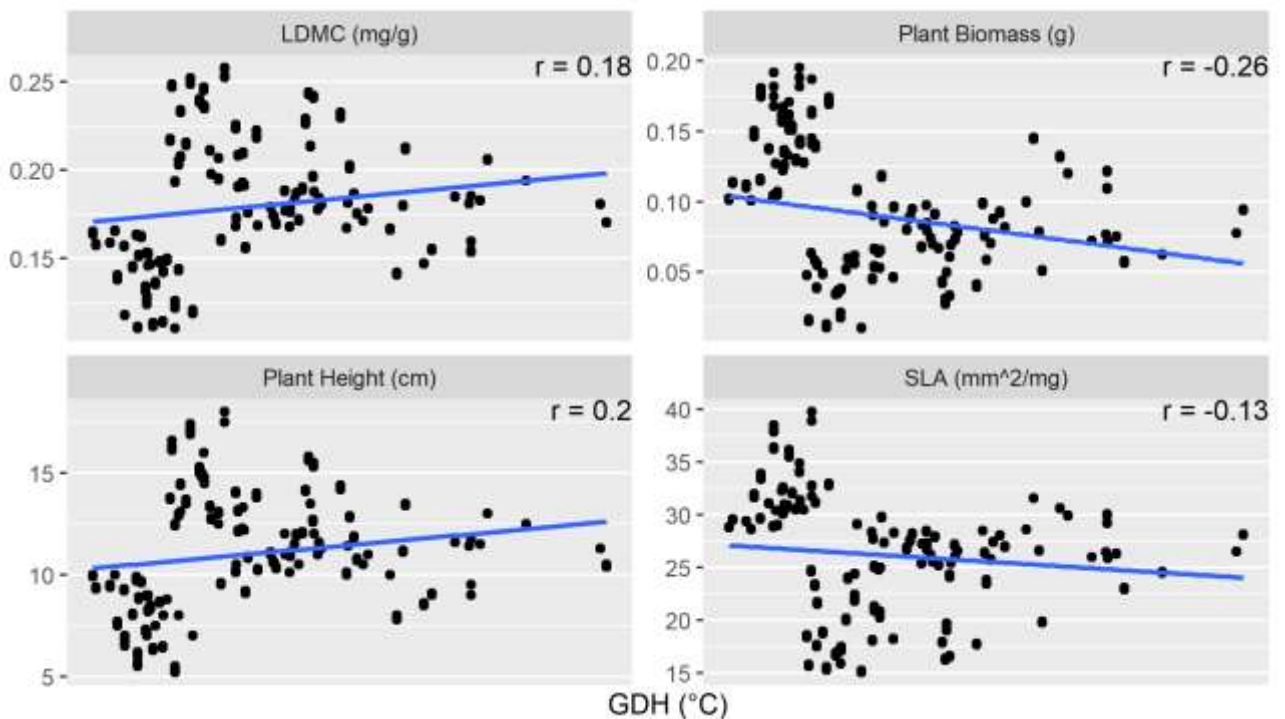


FIGURE 11 A, B, C, D: THE EFFECT OF GDH ON LDMC (TOP LEFT), PLANT BIOMASS (TOP RIGHT) , PLANT HEIGHT, (BOTTOM LEFT) AND SLA (BOTTOM RIGHT).

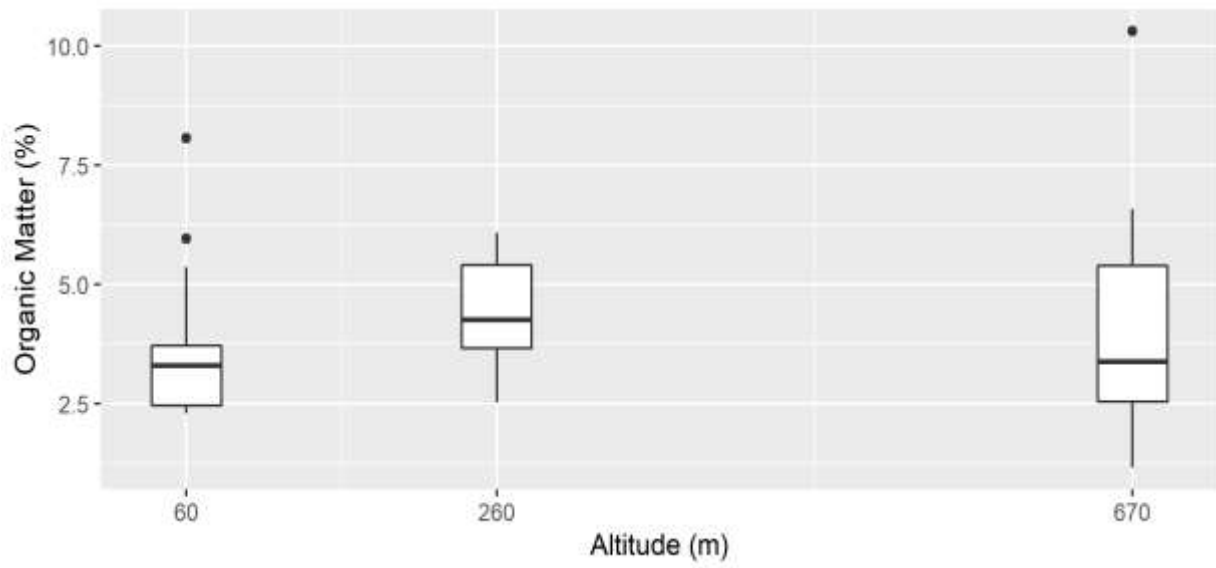


FIGURE 12 AVAILABILITY OF ORGANIC MATTER IN DIFFERENT ELEVATION.

4. Discussion

4.1 Effects of ³plot position, altitude and competition treatment on GDH and light

Different factors contributed to generate microclimatic difference along the elevation gradient and forest gradient. Our results clearly showed that accumulated temperature during the recorded growing season decreased the further away from the forest edge into the forest interior. More specifically, our result showed that GDH was strongly but negatively affected by plot distance from forest edge (figure 8 a and 8b). That means GDH decreased with increase in distance from the forest edge outside of the forest towards the interior of the forest. This is confirming the role of forest, mediating the microclimate experienced by understory plants, reducing air and soil temperature (De Frenne et al., 2013b). Competition also mattered for GDH but only at some altitude (Table 2). According, to my observation at different altitude, and site, forest was affected by the different factors. Overall, the lower elevation had low GDH, maybe because our sampling plots were very close to the ocean and cooler air from the ocean might have had cooling effect on the surrounding forest. In the mid-elevation, forests were quite open and in open forest, data loggers were more exposed to light and could have caused the logger chambers to be heated, might cause increased GDH. Secondly, these forests were steeper and south-east facing. These slopes most likely receive and accumulate higher light and temperature in the forest. In highest elevation (670m) the lowest GDH was recorded as expected (Körner, 2007).

Likewise, in my study mean-light intensity was negatively affected by plot distance, and competition from co-occurring species, (Table 1, figure 8). That means it is quite interesting that mean light intensity was quite higher outside of the forest and near to forest edge. Similarly, there was effect of competition for light. It is clear from (figure 8) that mean light value is lower in competition plots. Because light intensity reduces both due to overstory canopy trees inner side of the forest and co-occurring herbaceous species in competition plots. The effect of the competition from the herbaceous layer, however, decreased when moving into the forest interior. Similarly, the effect of competition also decreased along elevation.

4.2 Effect of elevation gradient on *A. nemorosa* trait

I expected the *A. nemorosa* show higher performance at higher elevation and our results indicated that *Anemone* attained greater height at higher elevation (Figure 11c). The greater height at higher elevation might be *Anemone* strategy to receive higher light than neighbouring species. Even though

³ Plot position, plot distance and forest gradient are interchangeable word are used throughout the work.

it is widely recognized that plant height decreases as elevation increases, especially at tree lines. Vellend and Geber, (2005) also reported *Urtica dioica*, a forest understory slow colonizing herb, which attained a greater plant height due to higher resource availability such as soil phosphorus and due to open canopies. Similarly, De Frenne et al., (2011a) reported taller plants at higher latitude and explained that increased plant height could be a higher resource acquisitive strategy of plants. Xu et al., (2017) also reported taller and wider plants at higher altitudes and suggested that severe human disturbance had a negative effect on the growth of the plant height at a lower elevation. Previous studies suggested that different factors determine the plant height along elevation gradients. For instance, Mao et al., (2018) reported that water availability is one of the key limiting factors for plant height at a species level because higher precipitation positively affects the plant height. However, we did not consider the presence of soil moisture or precipitation or other edaphic factors (Soil N,P) which may be another reason to contribute to *Anemone*'s height along an elevation gradient (Junker and Larue-Kontić, 2018).

Plant biomass can be used as an alternative for plant response to harsh conditions in high elevation (Ma et al., 2010). Because plant biomass is an important approach for understanding the response of individuals to local climate change along an elevation gradient (Ma et al., 2010). In my study I found decreased plant biomass with increased plant height at higher elevation which was quite surprising (Figure 11 b). My result about plant height and biomass is contradictory to several studies. However, the reason for decreased plant biomass in my sampling plots may be the presence of a lower amount of nutrient contents in plant tissues (He et al., 2017) which is indicated by (Figure 13) presence of the lowest organic matter at higher altitude. Similarly, at higher altitudes taller plants may produce smaller and thinner leaves which could be a reason for the reduced biomass. However, (Yüksek, (2009); and Yüksek et al., (2010) reported greater herbaceous biomass at higher altitude, but in low nutrient conditions. Furthermore, He et al., (2017), reported that, at higher elevation the reduced temperature and short growing season length limit the individual plant biomass which might be a reason for the negative correlation between plant height and plant biomass in my study.

SLA was negatively affected by the elevation gradient. Hence, lower elevations had higher SLA than higher elevations (Figure 11 d). Our results supported the finding from many other studies (Ackerly et al., 2002; Milla and Reich, 2011; Scheepens et al., 2010) indicating a response to decreasing temperature and increasing radiation, temperature, irradiance and water availability (Poorter et al., 2009). The availability of low organic matter, at higher elevation (Figure 13), may be a cause of producing leaves with lower SLA which is also supported by Bangroo et al., (2017). Shi et

al., (2018) also reported the positive correlation of soil organic matter and SLA. However, my results do not indicate any direct effect of organic matter on SLA. Furthermore, leaves with low SLA have higher LDMC which is beneficial to resist physical hazards such as e.g. herbivory, wind and hail. Which may be favourable traits of *A.nemorosa* to adapt in higher elevation. Therefore, it is clear that, taller *A. nemorosa* with lower SLA and high LDMC support the hypothesis that *A.nemorosa* perform better at elevation.

4.3 Effect of forest gradients on *A. nemorosa* traits

I expected the *A. nemorosa* show higher performance at forest interior. Because *Anemone* prefer to grow in forest canopy, cooler temperature and lower light available condition. Our results indicated that taller plants were outside of the forest with lower SLA (Figure 10 c, d) that is because outside of the forest, light intensity was higher. But at forest edge and near to forest edge plants were short with higher SLA because light intensity decreased at forest edge and neat to forest edge. Higher SLA under low light availability enhance light capturing capacity for higher photosynthesis (Liu et al., 2016).

4.4 Effect of competition on *A. nemorosa* traits

Plant competition are useful tools to explore the role of competition and its effects on the performance of competitors. Each individual, in a plant community either reduces the local resources which is called (competitive effect) and reduces resources tolerates by others (competitive response). Competition depressed individual performance can be evaluated with the trait-based performance (Navas and Violle, 2009). Here, I identified the effect of competition on functional traits of *A.nemorosa* in response to the elevation and forest gradient. My result clearly showed that there was direct effect of competition on *A. nemorosa* traits. From the (Table 3 and Figure 10), it is clear that, *A. nemorosa* were short in height than co-exist species at competition plots, but surprisingly, plant biomass was greater at competition plots; perhaps *Anemone* might produce produced bigger and heavier leaf. Therefore, *A. nemorosa* produced leaf with higher SLA in competition plots (Table3, figure11). Furthermore, there was strong competition outside of the forest than forest interior. That means due to strong competition, plant height was shorter at outside of the forest in competition plots. Likewise, as we got the opposite trend of plant height with plant biomass at higher elevation, similar trend exists in competition treatment along forest interior. Furthermore, in non-competition plots lower plant biomass with taller plant height may be due to the presence of lower amount of nutrients contents in plant tissues, or in non-competition plots taller plant might produce the small leaves which could be a reason to decreased biomass.

Another reason might be that; plant grew bigger in non-competition plots due to absence of other species and *A. nemorosa* might acquire higher amount of light and other soil resources (Soil N, or soil moisture). There was strong effect of interaction between altitude and competition treatment on *A. nemorosa* traits. Due to the interaction effect plant height were short in competition plots at elevation, but conversely, plant biomass and SLA were higher at competition plots. Therefore, my findings indicate that, *A. nemorosa* were superior competitors than coexisting species at higher elevation. Similarly, my findings demonstrated that, *A. nemorosa* were weak competitors at forest outside, forest edge and near to forest edge. But higher SLA at forest edge and near to forest edge indicate the well adaptive strategies of *A. nemorosa* in low light condition.

4.5 Effect of GDH and light on *A. nemorosa* traits

My third research question aimed to evaluate if microclimate (GDH and light) affects the functional traits of *A. nemorosa* along different elevation gradient. I clearly showed that GDH is negatively related to plant height and LDMC while GDH is positively related to plant biomass and SLA. Light had no direct effect on any traits of *A. nemorosa*. That means plant height and LDMC found to be reduced when accumulated temperature increased. However, previous studies (De Frenne et al., 2011a) showed that increased temperature advanced the regeneration and accelerated growth and height of two forest herbs *A. nemorosa* and *M. effusum* at higher latitude and in open top chamber experiment. Here, my results are the contrary of (De Frenne et al., 2011a) finding. Therefore, it is difficult to say how GDH affect to plant height but according to my results it is clear that *A. nemorosa* prefer to grow in lower temperature at higher elevation. However, the interaction of GDH and light, positively affected plant height and LDMC. While the interaction GDH and light negatively affected to SLA and plant biomass. Here I can argue that interaction of GDH and light and longer photoperiod in the growing season probably contributed to the plant height of *A. nemorosa*.

Rosbakh et al., (2015) reported similar findings, which also support my findings. They measured SLA of 29 grasslands species along the temperature gradient in Bavarian Alps and demonstrated a SLA–temperature relationship at the population (intraspecific), species (interspecific) and community level. Rosbakh et al., (2015) reported that 14 % of the studied species significantly reduced SLA along the temperature gradient. However, other study showed (Diaz et al., 1998; Diaz and Cabido, 1997) that temperature may not directly affect SLA but when it interacts with other factors such as soil fertility, soil moisture, disturbance and land-use types, it affects plant traits like plant height SLA and LDMC. Another example is; organic matter increases along the altitudinal

gradient but due to low temperature and high precipitation, their decomposition rate become slow and their availability become lower for plant (Yüksek, 2009; Yüksek et al., 2010). In this way temperature indirectly affect the plant traits via reducing soil nutrients. However, my result showed that lower organic matter (figure 13) and lower temperature in higher altitude could be a reason to produce lower SLA at higher elevation (Yüksek et al., 2010). Plants usually produce a leaf with higher SLA in low light intensity Reich et al., (2003), which is contrary to my results because in my study higher SLA were develop at low altitudes where the mean light intensity was lower than that at higher altitude.

5. Conclusion:

The output of this study clearly showed, that *Anemone*'s functional traits responded differently in different elevation and forest gradient and competition treatment. My result indicated that, *Anemone* functional traits are good indicator of detecting the impact of changing microenvironment in response to plant performance along elevation and forest gradient. At higher elevation, presence of taller anemone, indicates that they are adopting at higher elevation and performed better. At higher elevation, Presence of taller plant indicates the superior competitors for light acquisition than co-occurring species. *A.nemorosa* produce leaf with lower SLA leaf at higher elevation which indicates the well adaptive strategy in harsh environmental condition (like strong wind, irradiance and low temperature). Higher SLA near to forest edge, help plants to increase the efficiency of light capturing and maximize carbon gain in low light condition. Similarly, SLA and LDMC are associated with many critical aspects of plant growth and survival and involved in trade-off between LDMC production. Therefore, lower SLA produced with higher LDMC, which is also a favourable trait in higher elevation against the speedy wind and resource-poor condition. My results demonstrated that Competition has an effect on the traits of *A. nemorosa*. Forest outside and near to forest edge, there was strongest effect of competition. It seems that outside of the forest and near to forest edge *A. nemorosa* were weak competitors than co-occurring species, While, inner side of the forest competition effect became less. Overall, GDH affected to *A.nemorosa* traits but surprisingly direct light had no effect on any trait of *A.nemorosa*.

Bibliography

- Ackerly, D.D., Knight, C.A., Weiss, S.B., Barton, K., Starmer, K.P., 2002. Leaf size, specific leaf area and microhabitat distribution of chaparral woody plants: Contrasting patterns in species level and community level analyses. *Oecologia* 130, 449–457. <https://doi.org/10.1007/s004420100805>
- Alain F. Zuur Elena N. Ieno Graham M. Smith, 2007. *Analysing Statistical Data*.
- Antos, J.A., 2002. Understory plants in temperate forests, *Forests and forest plants - Encyclopedia of life support systems*.
- Bangroo, S.A., Najar, G.R., Rasool, A., 2017. Effect of altitude and aspect on soil organic carbon and nitrogen stocks in the Himalayan Mawer Forest Range. *Catena* 158, 63–68. <https://doi.org/10.1016/j.catena.2017.06.017>
- Bellemare, J., Moeller, D.A., 2015. Climate Change and Forest Herbs of Temperate Deciduous Forests, *The Herbaceous Layer in Forests of Eastern North America*. oxfordhb-9780199837656_c21.indd 462. <https://doi.org/10.1093/acprof:osobl/9780199837656.003.0021>
- Bramer, I., Anderson, B.J., Bennie, J., Bladon, A.J., De Frenne, P., Hemming, D., Hill, R.A., Kearney, M.R., Körner, C., Korstjens, A.H., Lenoir, J., Maclean, I.M.D., Marsh, C.D., Morecroft, M.D., Ohlemüller, R., Slater, H.D., Suggitt, A.J., Zellweger, F., Gillingham, P.K., 2018. Advances in Monitoring and Modelling Climate at Ecologically Relevant Scales, in: *Advances in Ecological Research*. Academic Press Inc., pp. 101–161. <https://doi.org/10.1016/bs.aecr.2017.12.005>
- Chen, J., Saunders, S.C., Crow, T.R., Naiman, R.J., Brosofske, K.D., Mroz, G.D., Brookshire, B.L., Franklin, J.F., 1999. Microclimate in Forest Ecosystem and Landscape Ecology. *Bioscience* 49, 288–297. <https://doi.org/10.2307/1313612>
- Cornelissen, J.H.C., Lavorel, S., Garnier, E., Díaz, S., Buchmann, N., Gurvich, D.E., Reich, P.B., Ter Steege, H., Morgan, H.D., Van Der Heijden, M.G.A., Pausas, J.G., Poorter, H., 2003. A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Aust. J. Bot.* <https://doi.org/10.1071/BT02124>
- De Frenne, P., Brunet, J., Shevtsova, A., Kolb, A., Graae, B.J., Chabrierie, O., Cousins, S.A., Decocq, G., De Schrijver, A., Diekmann, M., Gruwez, R., Heinken, T., Hermy, M., Nilsson, C., Stanton, S., Tack, W., Willaert, J., Verheyen, K., 2011a. Temperature effects on forest herbs assessed by warming and transplant experiments along a latitudinal gradient. *Glob. Chang. Biol.* 17, 3240–3253. <https://doi.org/10.1111/j.1365-2486.2011.02449.x>
- De Frenne, P., Brunet, J., Shevtsova, A., Kolb, A., Graae, B.J., Chabrierie, O., Cousins, S.A., Decocq, G., De Schrijver, A., Diekmann, M., Gruwez, R., Heinken, T., Hermy, M., Nilsson, C., Stanton, S., Tack, W., Willaert, J., Verheyen, K., 2011b. Temperature effects on forest herbs assessed by warming and transplant experiments along a latitudinal gradient. *Glob. Chang. Biol.* 17, 3240–3253. <https://doi.org/10.1111/j.1365-2486.2011.02449.x>
- De Frenne, Pieter, de Schrijver, A., Graae, B.J., Gruwez, R., Tack, W., Vandeloos, F., Hermy, M., Verheyen, K., 2010. The use of open-top chambers in forests for evaluating warming effects on herbaceous understorey plants. *Ecol. Res.* 25, 163–171. <https://doi.org/10.1007/s11284-009-0640-3>
- De Frenne, P., Graae, B.J., Brunet, J., Shevtsova, A., Schrijver, A. De, Chabrierie, O., Cousins, S.A.O., Decocq, G., Diekmann, M., Hermy, M., Heinken, T., Kolb, A., Nilsson, C., Stanton,

- S., Verheyen, K., 2012. The response of forest plant regeneration to temperature variation along a latitudinal gradient. *Ann. Bot.* 109, 1037–1046. <https://doi.org/10.1093/aob/mcs015>
- De Frenne, P., Graae, B.J., Kolb, A., Brunet, J., Chabrierie, O., Cousins, S.A.O., Decocq, G., Dhondt, R., Diekmann, M., Eriksson, O., Heinken, T., Hermy, M., Jõgar, Ü., Saguez, R., Shevtsova, A., Stanton, S., Zindel, R., Zobel, M., Verheyen, K., 2010. Significant effects of temperature on the reproductive output of the forest herb *Anemone nemorosa* L. *For. Ecol. Manage.* 259, 809–817. <https://doi.org/10.1016/j.foreco.2009.04.038>
- De Frenne, P., Rodríguez-Sánchez, F., Coomes, D.A., Baeten, L., Verstraeten, G., Vellen, M., Bernhardt-Römermann, M., Brown, C.D., Brunet, J., Cornelis, J., Decocq, G.M., Dierschke, H., Eriksson, O., Gilliam, F.S., Hédli, R., Heinken, T., Hermy, M., Hommel, P., Jenkins, M.A., Kelly, D.L., Kirby, K.J., Mitchell, F.J.G., Naaf, T., Newman, M., Peterken, G., Petřík, P., Schultz, J., Sonnier, G., Van Calster, H., Waller, D.M., Walther, G.R., White, P.S., Woods, K.D., Wulf, M., Graae, B.J., Verheyen, K., 2013a. Microclimate moderates plant responses to macroclimate warming. *Proc. Natl. Acad. Sci. U. S. A.* 110, 18561–18565. <https://doi.org/10.1073/pnas.1311190110>
- De Frenne, P., Rodríguez-Sánchez, F., Coomes, D.A., Baeten, L., Verstraeten, G., Vellen, M., Bernhardt-Römermann, M., Brown, C.D., Brunet, J., Cornelis, J., Decocq, G.M., Dierschke, H., Eriksson, O., Gilliam, F.S., Hédli, R., Heinken, T., Hermy, M., Hommel, P., Jenkins, M.A., Kelly, D.L., Kirby, K.J., Mitchell, F.J.G., Naaf, T., Newman, M., Peterken, G., Petřík, P., Schultz, J., Sonnier, G., Van Calster, H., Waller, D.M., Walther, G.R., White, P.S., Woods, K.D., Wulf, M., Graae, B.J., Verheyen, K., 2013b. Microclimate moderates plant responses to macroclimate warming. *Proc. Natl. Acad. Sci. U. S. A.* 110, 18561–18565. <https://doi.org/10.1073/pnas.1311190110>
- Diaz, S., Cabido, M., 1997. Plant functional types and ecosystem function in relation to global change, *Journal of Vegetation Science*. <https://doi.org/10.2307/3237198>
- Diaz, S., Cabido, M., Casanoves, F., 1998. Plant functional traits and environmental filters at a regional scale. *J. Veg. Sci.* 9, 113–122. <https://doi.org/10.2307/3237229>
- Ehrlén, J., Morris, W.F., 2015. Predicting changes in the distribution and abundance of species under environmental change. *Ecol. Lett.* 18, 303–314. <https://doi.org/10.1111/ele.12410>
- Falster, D.S., Westoby, M., 2003. Plant height and evolutionary games. *Trends Ecol. Evol.* [https://doi.org/10.1016/S0169-5347\(03\)00061-2](https://doi.org/10.1016/S0169-5347(03)00061-2)
- Gaston, K.J., 2003. *The Structure and Dynamics of Geographic Ranges*, Oxford Series in Ecology and Evolution. Oxford University Press. <https://doi.org/10.2167/jost191b.0>
- Givnish, T.J., 1988. Adaptation to sun and shade: a whole-plant perspective, *Australian Journal of Plant Physiology*.
- Gommers, C.M.M., Visser, E.J.W., Onge, K.R.S., Voeselek, L.A.C.J., Pierik, R., 2013. Shade tolerance: When growing tall is not an option. *Trends Plant Sci.* <https://doi.org/10.1016/j.tplants.2012.09.008>
- Griffith, T.M., Sultan, S.E., 2005. Shade tolerance plasticity in response to neutral vs green shade cues in *Polygonum* species of contrasting ecological breadth. *New Phytol.* 166, 141–148. <https://doi.org/10.1111/j.1469-8137.2004.01277.x>
- He, J. dong, Xue, J. yue, Gao, J., Wang, J. niu, Wu, Y., 2017. Adaptations of the floral characteristics and biomass allocation patterns of *Gentiana hexaphylla* to the altitudinal

- gradient of the eastern Qinghai-Tibet Plateau. *J. Mt. Sci.* 14, 1563–1576.
<https://doi.org/10.1007/s11629-017-4424-x>
- Honnay, O., Verheyen, K., Hermy, M., 2002. Permeability of ancient forest edges for weedy plant species invasion. *For. Ecol. Manage.* 161, 109–122. [https://doi.org/10.1016/S0378-1127\(01\)00490-X](https://doi.org/10.1016/S0378-1127(01)00490-X)
- Junker, R.R., Larue-Kontić, A.-A.C., 2018. Elevation predicts the functional composition of alpine plant communities based on vegetative traits, but not based on floral traits. *Alp. Bot.* 128, 13–22. <https://doi.org/10.1007/s00035-017-0198-6>
- Konare, H., Yost, R.S., Doumbia, M., Mccarty, G.W., Jarju, A., Kablan, R., 2010. Loss on ignition: Measuring soil organic carbon in soils of the sahel, west africa [WWW Document]. *African J. Agric. Res.* URL https://www.researchgate.net/publication/233981675_Loss_on_ignition_Measuring_soil_organic_carbon_in_soils_of_the_Sahel_West_Africa (accessed 11.15.19).
- Körner, C., 2007. The use of “altitude” in ecological research. *Trends Ecol. Evol.* 22, 569–574. <https://doi.org/10.1016/j.tree.2007.09.006>
- Körner, C., Hiltbrunner, E., 2018. The 90 ways to describe plant temperature. *Perspect. Plant Ecol. Evol. Syst.* <https://doi.org/10.1016/j.ppees.2017.04.004>
- Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2017. lmerTest Package: Tests in Linear Mixed Effects Models. *J. Stat. Softw.* 82. <https://doi.org/10.18637/jss.v082.i13>
- Lavorel, S., Garnier, E., 2002. Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Funct. Ecol.* 16, 545–556. <https://doi.org/10.1046/j.1365-2435.2002.00664.x>
- Lemke, I.H., Kolb, A., Graae, B.J., De Frenne, P., Acharya, K.P., Blandino, C., Brunet, J., Chabrierie, O., Cousins, S.A.O., Decocq, G., Heinken, T., Hermy, M., Liira, J., Schmucki, R., Shevtsova, A., Verheyen, K., Diekmann, M., 2015. Patterns of phenotypic trait variation in two temperate forest herbs along a broad climatic gradient. *Plant Ecol.* 216, 1523–1536. <https://doi.org/10.1007/s11258-015-0534-0>
- Liu, Y., Dawson, W., Prati, D., Haeuser, E., Feng, Y., Van Kleunen, M., 2016. Does greater specific leaf area plasticity help plants to maintain a high performance when shaded? *Ann. Bot.* 118, 1329–1336. <https://doi.org/10.1093/aob/mcw180>
- Ma, W.L., Shi, P.L., Li, W.H., He, Y.T., Zhang, X.Z., Shen, Z.X., Chai, S.Y., 2010. Changes in individual plant traits and biomass allocation in alpine meadow with elevation variation on the Qinghai-Tibetan Plateau. *Sci. China Life Sci.* 53, 1142–1151. <https://doi.org/10.1007/s11427-010-4054-9>
- Mao, L., Chen, S., Zhang, J., Zhou, G., 2018. Altitudinal patterns of maximum plant height on the Tibetan Plateau. *J. Plant Ecol.* 11, 85–91. <https://doi.org/10.1093/jpe/rtw128>
- McBratney, A.B., Odeh, I.O.A., Bishop, T.F.A., Dunbar, M.S., Shatar, T.M., 2000. Measuring pH of Soils. *Geoderma* 97, 293–327.
- Milla, R., Reich, P.B., 2011. Multi-trait interactions, not phylogeny, fine-tune leaf size reduction with increasing altitude. *Ann. Bot.* 107, 455–465. <https://doi.org/10.1093/aob/mcq261>
- Moles, A.T., Warton, D.I., Warman, L., Swenson, N.G., Laffan, S.W., Zanne, A.E., Pitman, A., Hemmings, F.A., Leishman, M.R., 2009. Global patterns in plant height. *J. Ecol.* 97, 923–932.

<https://doi.org/10.1111/j.1365-2745.2009.01526.x>

- Moser, G., Hertel, D., Leuschner, C., 2007. Altitudinal change in LAI and stand leaf biomass in tropical montane forests: A transect study in Ecuador and a pan-tropical meta-analysis. *Ecosystems* 10, 924–935. <https://doi.org/10.1007/s10021-007-9063-6>
- Navas, M.L., Violle, C., 2009. Plant traits related to competition: How do they shape the functional diversity of communities? *Community Ecol.* <https://doi.org/10.1556/ComEc.10.2009.1.15>
- Parmesan, C., Yohe, G., 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421, 37–42. <https://doi.org/10.1038/nature01286>
- Pickering, C.M., Venn, S., 2013. Increasing the Resilience of Australian Alpine Flora to Climate Change and Associated Threats: A Plant Functional Traits Approach.
- Poorter, H., De Jong, R., 1999. A comparison of specific leaf area, chemical composition and leaf construction costs of field plants from 15 habitats differing in productivity. *New Phytol.* 143, 163–176. <https://doi.org/10.1046/j.1469-8137.1999.00428.x>
- Poorter, H., Niinemets, Ü., Poorter, L., Wright, I.J., Villar, R., 2009. Causes and consequences of variation in leaf mass per area (LMA): A meta-analysis. *New Phytol.* <https://doi.org/10.1111/j.1469-8137.2009.02830.x>
- Poorter, L., 2007. Are species adapted to their regeneration niche, adult niche, or both? *Am. Nat.* 169, 433–442. <https://doi.org/10.1086/512045>
- Poorter, L., Castilho, C. V., Schiatti, J., Oliveira, R.S., Costa, F.R.C., 2018. Can traits predict individual growth performance? A test in a hyperdiverse tropical forest. *New Phytol.* 219, 109–121. <https://doi.org/10.1111/nph.15206>
- Reich, A.P.B., Wright, I.J., Bares, J.C., Craine, J.M., Oleksyn, J., Walters, M.B., Journal, I., Reich, P.B., Westoby, M., 2003. The Evolution of Plant Functional Variation: Traits, Spectra, and Strategies Reviewed work (s): Source: *International Journal of Plant Sciences*, Vol. 164, No. S3, Evolution of Functional Traits in Plants (May 2003), pp. S143–S164 Published by Evolution (N. Y). 164, S143–S164.
- Rosbakh, S., Römermann, C., Poschlod, P., 2015. Specific leaf area correlates with temperature: new evidence of trait variation at the population, species and community levels. *Alp. Bot.* 125, 79–86. <https://doi.org/10.1007/s00035-015-0150-6>
- Scheepens, J.F., Frei, E.S., Stöcklin, J., 2010. Genotypic and environmental variation in specific leaf area in a widespread Alpine plant after transplantation to different altitudes. *Oecologia* 164, 141–150. <https://doi.org/10.1007/s00442-010-1650-0>
- Scheepens, J. F., Frei, E.S., Stöcklin, J., 2010. Genotypic and environmental variation in specific leaf area in a widespread Alpine plant after transplantation to different altitudes. *Oecologia* 164, 141–150. <https://doi.org/10.1007/s00442-010-1650-0>
- Shi, H., Wen, Z., Guo, M., 2018. Leaf trait variation with environmental factors at different spatial scales: A multilevel analysis across a forest-steppe transition. *Forests* 9, 1–13. <https://doi.org/10.3390/f9030122>
- Shirreffs, D.A., 1985. *Anemone nemorosa* L. *J. Ecol.* 73, 1005. <https://doi.org/10.2307/2260164>
- Stevens, J.T., Safford, H.D., Harrison, S., Latimer, A.M., 2015. Forest disturbance accelerates thermophilization of understory plant communities. *J. Ecol.* 103, 1253–1263. <https://doi.org/10.1111/1365-2745.12426>

- Stocker, T.F., Qin, D., Plattner, G.K., Tignor, M.M.B., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M., 2013. Climate change 2013 the physical science basis: Working Group I contribution to the fifth assessment report of the intergovernmental panel on climate change, Climate Change 2013 the Physical Science Basis: Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. <https://doi.org/10.1017/CBO9781107415324>
- Team, R.C., 2019. The R Project for Statistical Computing [WWW Document]. <Http://Www.R-Project.Org/>. URL <https://www.r-project.org/> (accessed 11.11.19).
- Valladares, F., Niinemets, Ü., 2008. Shade Tolerance, a Key Plant Feature of Complex Nature and Consequences. *Annu. Rev. Ecol. Evol. Syst.* 39, 237–257. <https://doi.org/10.1146/annurev.ecolsys.39.110707.173506>
- Vanneste, T., Michelsen, O., Graae, B.J., Kyrkjeeide, M.O., Holien, H., Hassel, K., Lindmo, S., Kapás, R.E., De Frenne, P., 2017. Impact of climate change on alpine vegetation of mountain summits in Norway. *Ecol. Res.* 32, 579–593. <https://doi.org/10.1007/s11284-017-1472-1>
- Vellend, M., Geber, M.A., 2005. Connections between species diversity and genetic diversity. *Ecol. Lett.* 8, 767–781. <https://doi.org/10.1111/j.1461-0248.2005.00775.x>
- Violle, C., Navas, M.L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., Garnier, E., 2007. Let the concept of trait be functional! *Oikos* 116, 882–892. <https://doi.org/10.1111/j.2007.0030-1299.15559.x>
- Walther, G., Beißner, S., Burga, C.A., 2005. Trends in the upward shift of alpine plants. *J. Veg. Sci.* 16, 541–548. <https://doi.org/10.1111/j.1654-1103.2005.tb02394.x>
- Westoby, M., 1998. A leaf-height-seed (LHS) plant ecology strategy scheme. *Plant Soil* 199, 213–227. <https://doi.org/10.1023/A:1004327224729>
- Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J.H.C., Diemer, M., Flexas, J., Garnier, E., Groom, P.K., Gulias, J., Hikosaka, K., Lamont, B.B., Lee, T., Lee, W., Lusk, C., Midgley, J.J., Navas, M.-L., Niinemets, U., Oleksyn, J., Osada, N., Poorter, H., Poot, P., Prior, L., Pyankov, V.I., Roumet, C., Thomas, S.C., Tjoelker, M.G., Veneklaas, E.J., Villar, R., 2004. The worldwide leaf economics spectrum. *Nature* 428, 821–7. <https://doi.org/10.1038/nature02403>
- Xu, M., Ma, L., Jia, Y., Liu, M., 2017. Integrating the effects of latitude and altitude on the spatial differentiation of plant community diversity in a mountainous ecosystem in China. <https://doi.org/10.1371/journal.pone.0174231>
- Yüksek, F., Altun, L., Karaöz, Ö., Şengönül, K., Yüksek, T., Küçük, M., 2013. The Effect of Altitude on Soil Properties and Leaf Traits in Wild *Vaccinium arctostaphylos* L. Populations in the Forest Understory in Firtina River Basin. *Int. Cauc. For. Symp.* 577–583.
- Yüksek, T., 2009. Effect of visitor activities on surface soil environmental conditions and aboveground herbaceous biomass in Ayder Natural Park. *Clean - Soil, Air, Water* 37, 170–175. <https://doi.org/10.1002/clen.200700208>
- Yüksek, T., Kurdoğlu, O., Yüksek, F., 2010. The effects of land use changes and management types on surface soil properties in Kafkasör protected area in Artv Turkey. *L. Degrad. Dev.* 21, 582–590. <https://doi.org/10.1002/ldr.1000>
- Zhang, Z., Papaik, M.J., Wang, X., Hao, Z., Ye, J., Lin, F., Yuan, Z., 2016. The effect of tree size, neighborhood competition and environment on tree growth in an old-growth temperate forest.

