UNIVERSITY OF NAIROBI

EVALUATION OF PHENOTYPIC PLASTICITY IN REPRODUCTIVE FITNESS TRAITS OF *Arabis alpina* L. (BRASSICACEAE) IN THE AFROMONTANE REGION OF MOUNT KENYA

BY

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DECLARATION

I declare that this thesis is my original work and has not been submitted elsewhere for examination or the award of a degree. Where other people's work has been used, it has been properly acknowledged and referenced.

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DEDICATION

I dedicate this study to my lovely children, my wife, my parents, and my former boss and mentor, the late Dr. Patrick N. Muthoka, with heartfelt gratitude and love.

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ABSTRACT

The Afromontane region is widely recognized as a biodiversity hotspot, harboring rare, endemic, and threatened plant species. However, these species face multiple threats, including habitat fragmentation, deforestation, invasive species, overgrazing, agricultural expansion, and the impact of climate change. Climate change poses a significant environmental challenge in the region, resulting in rising temperatures and changing precipitation patterns. Consequently, the Afromontane flora has developed adaptations to cope with these fluctuating climatic conditions. These adaptations result in changes in species distribution, alterations in plant-animal interactions, increased vulnerability to pest and disease outbreaks, and variations in fitness parameters such as phenology, seed production, germination, and survival rates. Despite the ecological significance of this ecosystem and the potential impacts of climate change, our understanding of how its flora responds to these selective pressures remains limited. Additionally, there is a scarcity of studies investigating the influence of soil properties on the variability of reproductive fitness traits within, between, and among tropical Afromontane species. Although there have been studies exploring the effects of climate change on various aspects of plant biology, research specifically examining the impact of changing climatic factors on the germination and seedling survival rates of tropical Afromontane species is lacking. This study addresses these research gaps by assessing the phenotypic plasticity of reproductive fitness traits in *Arabis alpina* L. (Brassicaceae) in the Afromontane region of Mt. Kenya. It examines the impact of elevation and soil characteristics on the reproductive fitness traits of *A. alpina*, explores potential trade-offs among these traits, and investigates the influence of seed trait selection on its germination and survival rates. The populations of *A. alpina* were sampled using the belt transect method. Twenty-eight sample plots of *A. alpina* were identified, covering an altitude range of 3,763m to 4,600m. The onset and duration of flowering in *A. alpina* individuals were monitored visually. Seeds of the species were collected, processed, counted, weighed, and subjected to germination trials and seedling survival counts. Soil samples were randomly collected from the sample plots, and soil characteristics such as carbon, nitrogen, phosphorus, pH, and electrical conductivity were analyzed. Statistical analyses were performed using R version 4.3.1. A one-way analysis of variance was used to assess variations in the reproductive fitness traits of *A. alpina* across lower alpine, upper alpine, and nival vegetation zones. In addition, linear mixed-effects models were employed to determine variations in the reproductive fitness traits of *A. alpina* along the elevation gradient and establish the degree of association between these traits.

A model convergence analysis was performed to evaluate the performance and accuracy of the models in estimating the response variables. The results indicate that soil nitrogen, carbon, phosphorus, and pH decrease while soil electrical conductivity increases with elevation. These variations in soil characteristics significantly contribute to the observed differences in the onset and duration of flowering, seed mass, seed number, germination, and seedling survival rates of *A. alpina* across vegetation zones and along the elevation gradient. Specifically, with every 100-meter increase in elevation, there is a 19-hour and 20-minute delay in the onset of flowering, a reduction of 1 day and 17 hours in the species' duration of flowering, a decrease of 0.4 mg in seed mass, an increase of 7 seeds, and an 8% decrease in germination and seedling survival rates. Trade-offs were observed among the reproductive fitness traits of *A. alpina* along the elevation gradient. As the elevation increases, a one-day delay in the species' onset of flowering results in a reduction of its duration of flowering by 1 hour and 41 minutes. Moreover, a decrease of 1 mg in the species' seed mass corresponds to an increase of 8 seeds. Furthermore, a 1% decrease in the species' germination rate leads to a 0.82% decrease in its seedling survival rate. Different seed resource allocation strategies have varying effects on the germination and survival rates of *A. alpina*. Particularly, a 1 mg increase in *A. alpina* seed mass leads to a 9.1% increase in its germination rate and an 8.5% increase in its seedling survival rate. In contrast, an increase of 1 seed results in a 0.88% decrease in the germination rate and a 0.78% decrease in the seedling survival rate of the species. These plastic responses of *A. alpina* in response to changing climatic conditions along the elevation gradient underscore the adaptive strategies employed by the species to thrive in diverse ecological niches within the Afromontane ecosystem of Mt. Kenya. This understanding will assist in predicting the future distribution and population dynamics of not only this species but also others in comparable environments. By identifying the underlying mechanisms of phenotypic plasticity and adaptive responses of Afromontane flora to environmental changes, conservation strategies can be developed to safeguard biodiversity and protect threatened species amidst the ongoing challenges posed by climate change.

Keywords: climate change, elevation, germination, phenology, seed traits, seedling survival

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LIST OF ABBREVIATIONS AND ACRONYMS

%	Percentage
&	and
$(NH_4)_2MoO_4$	Ammonium molybdate
$\equiv$	Equivalent to
°C	Degree Celsius
a.s.l	Above Sea Level
Alt. range	Altitudinal range
AM	Ante Meridiem
ANOVA	Analysis of Variance
ATP	Adenosine triphosphate
C	Carbon
C_7H_8	Toluene
$CaCl_2$	Calcium chloride
CO_2	Carbon dioxide
conc.	concentrated
df	Degree of freedom
DI	Deionized
dS/m	deciSiemens per metre
EC	Electrical Conductivity
H_0	Null hypothesis
H_2S0_4	Sulphuric Acid
H_3BO_3	Boric acid.
H_3PO_4	Orthophosphoric Acid
HCl	Hydrochloric Acid
KCl	Potassium Chloride
KH_2PO_4	Monobasic Potassium Phosphate
$KSbOC_4HO_4$	Potassium Antimony Tartrate
LSD	Least Significant Difference
mg	milligram
mL	millilitres
mm	millimeter
Mt	Mount
N	Nitrogen
NH_2S0_4	Ammonium sulfite
P	Phosphorus
PCA	Principal Component Analysis
pH	Potential for Hydrogen
PM	Post Meridiem
ppm	Parts per million
Q-Q	Quartile-Quartile
R^2_{adj}	Adjusted R-squared
RSE	Residual standard error
UNESCO	United Nations Educational, Scientific and Cultural Organization
UV	Ultra-violet
μ	Average

CHAPTER ONE: INTRODUCTION

1.1. Background Information

The Tropical Eastern Afromontane region is globally recognized as one of the 35 biodiversity hotspots, harboring a remarkable diversity of rare, endemic, and threatened species (Conservation International, 2023). This region is distinguished into four main vegetation zones: montane, ericaceous, moorland, and alpine zones (White, 1983; Gebremedhin *et al*., 2021). These zones are shaped by altitude, temperature, and rainfall (Salick *et al*., 2014). They provide a habitat for unique species that cannot be found elsewhere globally (Hedberg, 1964). These species are adapted to endure high ultraviolet radiation, nutrient-poor soils, temperature fluctuations, and strong winds.

1.2. Statement of the Research Problem

Climate change presents a significant environmental challenge in the region, leading to rising temperatures and shifting precipitation patterns. Consequently, plants in this region adapt by undergoing distribution shifts, modifying plant-animal interactions, and adjusting resource allocation strategies to enhance fitness traits like phenology, seed production, germination, and seedling survival rates (Williams *et al*., 2007; Hovenden *et al*., 2008; King *et al*., 2012). Nevertheless, our understanding of how the flora in the Afromontane ecosystem responds to these selective pressures remains limited.

Variations in these fitness traits have been associated with elevation-dependent environmental factors, including soil nutrients (Yeşilyurt *et al*., 2017; Chen *et al*., 2018). These factors determine the energy available for reproduction and seedling survival (Moles *et al*., 2004; Li *et al*., 2023). Nevertheless, there is a lack of research investigating the effects of soil properties on the variability of reproductive fitness traits within, between, and among tropical Afromontane species (Kołodziejek, 2017; Chen *et al*., 2018).

Germination and seedling survival rates are vital indicators of a species' capacity to endure and reproduce in dynamic environmental conditions. These rates are influenced by soil parameters, temperature, precipitation, soil moisture, and other climatic variables. Numerous studies have investigated the effects of climate change on different aspects of plant biology. However, research specifically examining the impact of changing environmental factors on germination and seedling survival rates of tropical Afromontane species is lacking.

To address these research gaps, this study evaluated the phenotypic plasticity of reproductive fitness traits in *Arabis alpina* in the Afromontane region of Mount Kenya. The findings from this study will enhance our comprehension of the adaptive strategies employed by *A. alpina* in response to fluctuating environmental factors along the elevation gradient. This knowledge is essential for developing effective conservation strategies and management approaches to protect and preserve these unique species.

1.3. Objectives of the Study

1.3.1. General Objective

This study assessed the phenotypic plasticity of reproductive fitness traits in *Arabis alpina* L. (Brassicaceae) in the Afromontane region of Mount Kenya.

1.3.2. Specific Objectives

1) To investigate whether the reproductive fitness traits of *A. alpina* exhibit a plastic response to changes in elevation and soil characteristics.
2) To examine whether the reproductive fitness traits of *A. alpina* exhibit trade-offs in response to changes in elevation and soil characteristics.
3) To assess the influence of seed resource allocation strategies on the germination and seedling survival rates of *A. alpina* along the elevation gradient.

1.3.3. Hypotheses

1) **H_{01}**: The reproductive fitness traits of *A. alpina* do not exhibit any plastic response to changes in elevation and soil characteristics.
2) **H_{02}**: The reproductive fitness traits of *A. alpina* do not exhibit any trade-offs in response to changes in elevation and soil characteristics.
3) **H_{03}**: The selection of seed resource allocation strategies does not influence the germination and seedling survival rates of *A. alpina* along the elevation gradient.

1.4. Justification and Significance of the Study

Evaluating the phenotypic plasticity in reproductive fitness traits of *Arabis alpina* in response to elevation and soil physicochemical properties holds significant implications for our understanding of plant adaptation in dynamic ecosystems. Variable floral phenology responds to different environmental conditions within and/or between populations. These distinctions are critical for understanding natural selection and its influence on the evolution of floral traits. Only a few studies have documented natural variation in flowering times of plants due to climate-induced phenological alterations (Rafferty *et al*., 2013; Rafferty *et al*., 2016). As a result, limited knowledge exists regarding the variation in onset and duration of flowering (Bustamante & Búrquez, 2008) among populations of the same species (Li *et al*., 2021).

Assessing resource allocation strategies in response to the changing environmental conditions surrounding Afromontane plant species is a step towards understanding the selection pressures that may have contributed to their response, whether genetic or adaptive. This will aid in identifying suitable habitats for their growth and establishment, thus enhancing the seedling survival of these species.

Ultimately, studying the plastic responses of *Arabis alpina*, the study species, will:

1) Provide valuable information about its adaptive strategies and mechanisms of resilience.
2) Contribute to knowledge of the ecological processes shaping the diversity and distribution of species within the tropical Afromontane ecosystem.
3) Assess the range and magnitude of variation in traits exhibited by this species under different environmental conditions by measuring and quantifying its plastic responses. This provides a deeper understanding of the species' flexibility and adaptability.
4) Aid in predicting and managing the impact of climate change on plant populations and ecosystems. This will contribute to the development of conservation strategies that promote the resilience and survival of the species and its associated species in the face of ongoing environmental challenges.
5) Enhance our understanding of plant ecology, evolution, and survival strategies in dynamic environments like the Afromontane ecosystem.

CHAPTER TWO: LITERATURE REVIEW

2.1. Description of the Afromontane Ecosystem

2.1.1. Origin

The rising of mountains at different times from the early Miocene epoch to the late Pleistocene epoch led to the geographic isolation of Afromontane plants (Hedberg, 1961; Hedberg, 1970). Continuous glaciations during the Pleistocene of the Quaternary period altered the flora zonation along the Afromontane belt (Hedberg, 1970). This is evident by earlier glaciation traces at the terminal moraines of Mount Kenya at an altitude of 2900m (Gregory, 1894; Mackinder, 1900; Coe, 1969).

2.1.2. Climate

Temperature increases in the 21st century have been reported, especially in the mountainous ecosystem (Nogués-Bravo *et al.*, 2007; Pachauri & Mayer, 2014). In addition, this ecosystem is characterized by extreme ecological conditions resembling 'summer every day and winter every night' (Harris *et al.*, 2004). This means that temperatures rise rapidly in the morning, while fluctuations in wind and cloudiness lead to drastic temperature changes throughout the day. A combination of higher elevation, cooler temperatures, and increased precipitation gives rise to diverse vegetation zones. These zones serve as important refugia for rare, endemic, and threatened species, making them of significant conservation value.

2.1.3. Flora

This flora comprises a remarkable assemblage of plant species with unique morphological and physiological adaptations to the extreme Afromontane climatic conditions (Halloy, 1983; Chala *et al.*, 2016). Alpine species, such as the Giant Groundsel (*Dendrosenecio*), have developed adaptive modifications to thrive in harsh climatic conditions (Hedberg, 1964). These species, for example, possess large leaves that fold at night, providing protection against freeze-thaw cycles. Their apical meristems retain water, thereby mitigating thermal shocks caused by temperature fluctuations throughout the day and night. The intact old leaves attached to the plant act as insulation during cold nights (Smith, 1974; Hedberg & Hedberg, 1979; Chala *et al.*, 2016). Furthermore, their tall stature enables them to avoid temperature fluctuations at ground level (Fetene *et al.*, 1998; Jacob *et al.*, 2015). The rapid change in the vegetation structure and composition of this region can be attributed to reduced altitudinal ranges, which consequently lead to increased genetic drift, habitat loss, and increased vulnerability to climate change (Hedberg, 1970; Garcia *et al.*, 2000).

As the climate warms, this flora faces new selection pressures to alter its reproductive timing. These changes influence the ecological processes that determine the distribution of this flora (Telwala *et al*., 2013). As a result, mountain ecosystems are regarded as suitable observation sites for tracking the effects of climate change (Körner, 2003).

2.1.4. Conservation Importance

Afromontane ecosystems have remarkably high species richness and endemism, necessitating their protection to preserve unique species and genetic diversity. These ecosystems regulate water flow, maintain soil fertility, prevent erosion, and contribute to climate regulation, all of which are crucial for the well-being of local communities and sustainable resource use. Moreover, they mitigate climate change by acting as carbon sinks, absorbing and storing significant amounts of CO_2 from the atmosphere.

2.2. Reproductive Fitness Traits

2.2.1. Onset and Duration of Flowering

Phenology refers to the timing of an organism's biological events (Fitchett *et al*., 2015). The International Biological Programme defines it as "the study of recurrent biological events' timing, the causes of their timing concerning biotic and abiotic forces, and the interaction among phases of the same or different species" (Lieth, 1974).

The onset of flowering is the time (in days) between germination and the opening of the first flower (Helsen *et al*., 2020). Changes in elevation result in corresponding shifts in climatic conditions, which in turn affect the onset of flowering (Marquis, 1988; Harsch & Bader, 2011). Cooler temperatures at higher elevations may delay the onset of flowering (Archibold, 1995; Bertin, 2008). A study conducted by Fitter & Fitter (2002) reported changes in the onset of flowering in British plants as a result of temperature increases. These changes disrupt plant-pollinator interactions (Holtsford & Ellstrand, 1990; Stenseth & Mysterud, 2002; Visser & Both, 2005; Memmott *et al*., 2007; Both *et al*., 2009; Zhou *et al*., 2022), resulting in variations in seed set over time (Carpenter, 1976; Schemske, 1977; Schemske *et al*., 1978; Waser, 1978; Gross & Werner, 1983). Early- and late-flowering individuals experience different environmental conditions, leading to distinct selection pressures on their flowering phenology (Alexander *et al*., 2009). However, there is no consensus on the advantages and disadvantages of early- versus late-flowering species and individuals in the face of global warming (Giménez-Benavides *et al*., 2011).

The duration of flowering refers to the length of time a plant remains in bloom (Monson, 2014). It varies among species and is influenced by genetic factors, resource availability, temperature, precipitation, and day length changes (Dainese, 2012). Longer flowering periods enhance a plant's fitness by increasing the chances of attracting pollinators, leading to higher seed set and reproductive output (Rafferty *et al*., 2013). Additionally, it increases the chances of encountering suitable environmental conditions for pollination and seed development (Burkle & Irwin, 2009). It also allows plants to adapt to variations in temperature, light, and other environmental factors, increasing the likelihood of successful reproduction (Wu *et al*., 2018). On the other hand, a shorter duration of flowering may reduce the chances of attracting pollinators and limit the time available for successful pollination (Molau, 1993). This decreases a species' reproductive output and fitness. Therefore, studying flowering phenology shifts provides a measurable method to determine the ecological impact of climate change on the Afromontane flora (Van Vliet *et al*., 2003; Badeck *et al*., 2004; Cleland *et al*., 2012).

2.2.2. Seed Mass and Seed Number

Seed mass is the weight or size of an individual seed, while seed number is the quantity of seeds produced by a plant (Souza *et al*., 2019). These traits affect seed germination, establishment, and overall plant fitness (Ge *et al*., 2020). Variation in seed traits exists among individuals, species, and populations (Tremayne & Richards, 2000). This variation is primarily driven by environmental pressures during seed development that create a trade-off between seed mass and quantity per plant (Eriksson, 1999; Tremayne & Richards, 2000).

In mountain ecosystems, selection pressures shape resource allocation in plants (Moles *et al*., 2004). Seed mass and seed number are crucial parameters determining resource allocation strategies in response to climate change (Kery *et al*., 2000; Guo *et al*., 2010). Studies have established correlations between seed mass and elevation (Zhou *et al*., 2013; Hughes & Simons, 2014). Negative relationships between seed mass and elevation were observed in herbaceous species and populations in California (Ge *et al*., 2020). Guo *et al*. (2010) and Olejniczak *et al*. (2018) documented a seed mass reduction with increasing elevation. This can be attributed to lower photosynthetic rates at higher altitudes due to lower temperatures and a shorter growing season, limiting seed development and provisioning (Baker, 1972; Guo *et al*., 2010; Qi *et al*., 2014; Ge *et al*., 2020). Consequently, small and light seeds are favored for producing numerous offspring that can colonize the stressful high-elevation environments (Olejniczak *et al*., 2018).

A study on seed weight in the Swiss Alps (Pluess *et al*., 2005) reported a positive correlation between seed mass and elevation. This aligns with the 'stress tolerance' mechanism proposed by Boulli *et al*. (2001), Pluess *et al*. (2005), Bu *et al*. (2007), Guo *et al*. (2010), Wang *et al*. (2014), and Qi *et al*. (2015). In this mechanism, larger seeds are predicted to have better survival in stressful high-elevation environments. This finding is corroborated by a study on the reproduction strategy of *Chloris virgata* (Changfu & Ying, 2015). Conversely, a consistent relationship between seed mass and elevation was not observed across studies. A significant trade-off between seed mass and number was observed among species and populations (Chen *et al*., 2018). This aligns with a study on seed traits in *Ipomoea purpurea* (Mojonnier, 1998). Nevertheless, in a study on seed and fruit trade-offs in Amazon pioneers, genetic and phenotypic correlations between seed mass and number varied across different species (Bentos *et al*., 2014).

Selection pressures contribute to seed trait variations. Higher seed mass facilitates colonization of stressful habitats due to faster germination and a greater seedling survival rate (Mogie *et al*., 1990; Tremayne & Richards, 2000). Larger seeds may face increased predation costs. Increased seed weight in green grams supports the bean weevil eggs and thus increases their survival (Mitchell, 1975). Bruchid beetles prefer larger seeds over smaller ones for predation (Nelson & Johnson, 1983; Jordano, 1984; Morse & Schmitt, 1985). Resource limitation impedes seed provisioning and development, leading to the production of smaller seeds. This was observed in a study on the effects of resource limitations on the seed mass of *Banksia spinulosa* (Vaughton & Ramsey, 1997). Soil nutrient availability affects stigma-receptivity timing (Hersh *et al*., 2015; Lankinen & Hydbom, 2017). Early stigma receptivity in *Collinsia heterophylla* favours paternal fitness. Late stigma receptivity favours maternal fitness by increasing seed production (Lankinen & Hydbom, 2017).

2.3. 'Darwinian' Fitness

2.3.1. Seed Germination

This is the initial phase in the lifecycle of higher plants (Wolny *et al*., 2018), followed by the growth of the seedling (Rajjou *et al*., 2012). It varies among populations and is influenced by elevation (Ge *et al*., 2020). Moisture and temperature significantly affect seed germination (Roberts, 1988; Ge *et al*., 2020). Bu *et al*. (2007) reported improved germination in response to diurnal temperature fluctuations rather than constant temperatures. This leads to significant differences in germination rates among populations (Mira *et al*., 1970).

2.3.2. Seedling Survival

This is the percentage of seedlings in a population that withstand and persist under specific conditions (Klemow & Raynal, 1983; Ordoñez-Salanueva *et al*., 2017). It is influenced by resource competition, herbivory, disease, and abiotic stressors such as temperature extremes and drought (Hau & Corlett, 2003). Plants have evolved strategies to enhance seedling survival. Some have extensive roots that access water in deeper soil layers, enhancing drought resistance. Others shed leaves during dry periods to conserve water until favourable conditions arise (Bowman & Hacker, 2021). Certain species have reduced leaf surface area to minimize water loss in moisture-limited environments, as well as thick cuticles and waxy coatings on their leaves to protect against water loss and cold temperatures.

2.3.3. Effect of Seed Mass on Germinability and Survival

Seed mass has consistently been linked to both germination and seedling survival rates (Bu *et al*., 2007; Ge *et al*., 2020). A study on the effect of elevation and seed mass on the germinability of eight *Allium* species reported a positive correlation between seed mass, germination, and seedling survival rates (Ge *et al*., 2020). This suggests that larger seeds have a higher likelihood of successful germination and seedling survival (Lönnberg & Eriksson, 2013). The presence of larger energy reserves in bigger seeds likely confers advantages during germination and the establishment of early-sprouting seedlings. Additionally, it enhances access to crucial resources such as nutrients and water, ultimately promoting higher germination and seedling survival rates. In contrast, Counts & Lee (1991) and Bu *et al*. (2007) observed a negative correlation between seed mass, germination, and seedling survival rates. However, other studies by McKersie *et al*. (1981), Eriksson (1999), Greipsson (1995), Bu *et al*. (2007), Wang *et al*. (2009), and Larios *et al*. (2014) did not find any significant correlations among seed mass, germination, and seedling survival rates. These findings indicate that the relationship among seed mass, germination, and seedling survival rates may vary among different species and in different environments (Moles & Westoby, 2006; Cao *et al*., 2016).

2.4. Phenotypic Plasticity

2.4.1. Mechanisms of Phenotypic Plasticity

Phenotypic plasticity is achieved through developmental responses, physiological adjustments, and changes in gene expression. Developmental responses alter growth timing and influence flowering and seed production (Sultan, 2000).

Physiological adjustments involve modifying metabolic processes and resource allocation to adapt to different environments. For instance, limited resources can lead to increased resource allocation for seed development, resulting in larger or more numerous seeds (Schlichting, 1986). Changes in gene expression activate or deactivate specific genes based on environmental cues, producing traits that enhance reproductive fitness (Pigliucci *et al*., 2006).

2.4.2. Environmental Factors Influencing Phenotypic Plasticity

2.4.2.1. Elevation Gradient

Elevation is a crucial factor influencing phenotypic plasticity in plants (Gratani, 2014). As elevation increases, temperatures, oxygen, and UV radiation levels change (Peacock, 1998; Barnes *et al*., 2023). These environmental variations exert selective pressures on plants, leading to plastic responses in their reproductive fitness traits. For example, plants at higher elevations may delay flowering to avoid frost damage or adjust seed mass and number to cope with lower nutrient availability and reduced pollinator activity (Körner, 2003).

2.4.2.2. Soil Characteristics

Soil nutrient availability varies with altitude. High-elevation soils are less productive compared to low-elevation soils (Loomis *et al*., 2006; Huber *et al*., 2007; Wagg *et al*., 2011) due to snow accumulation patterns, microtopography, and nutrient decomposition (Seastedt *et al*., 2004; Holtmeier, 2009). In addition, Lower temperatures, cooler and wetter conditions, and lower organic matter contribute to lower soil fertility at higher elevations (Davis *et al*., 2018). Soil physicochemical properties shape species' responses, adaptation, community interactions, and conservation strategies (Han *et al*., 2022; Kudureti *et al*., 2023). Resource allocation strategies in plants vary under different soil conditions (Hu *et al*., 2021). Plants may adjust the timing and duration of flowering based on soil nutrient availability (Nord & Lynch, 2009; Cho *et al*., 2017). Seed mass and number can also vary in response to soil moisture content and nutrient availability (Zhang, 1995). Therefore, assessing the effects of soil properties on the phenology, seed traits, germination, and seedling survival rates of *Arabis alpina* is ecologically significant for evaluating its phenotypic plasticity.

2.4.3. Significance of Phenotypic Plasticity

Phenotypic plasticity enables plant populations and communities to cope with environmental variability, optimize resource allocation, and maximize reproductive success (Ghalambor *et al*., 2007; Forsman, 2015). Plants utilize phenotypic plasticity to adapt to varying elevation gradients and soil conditions (Gratani, 2014; Brooker *et al*., 2022).

CHAPTER THREE: MATERIALS AND METHODS

3.1. Description of the Study Area

3.1.1. Location

Mount Kenya is located in central Kenya, East Africa. The mountain is situated near the equator and is positioned between the coordinates 0°09' S latitude and 37°18' E longitude. It occupies an intersection area of six counties, namely: Nyeri, Meru, Laikipia, Embu, Kirinyaga, and Tharaka Nithi.

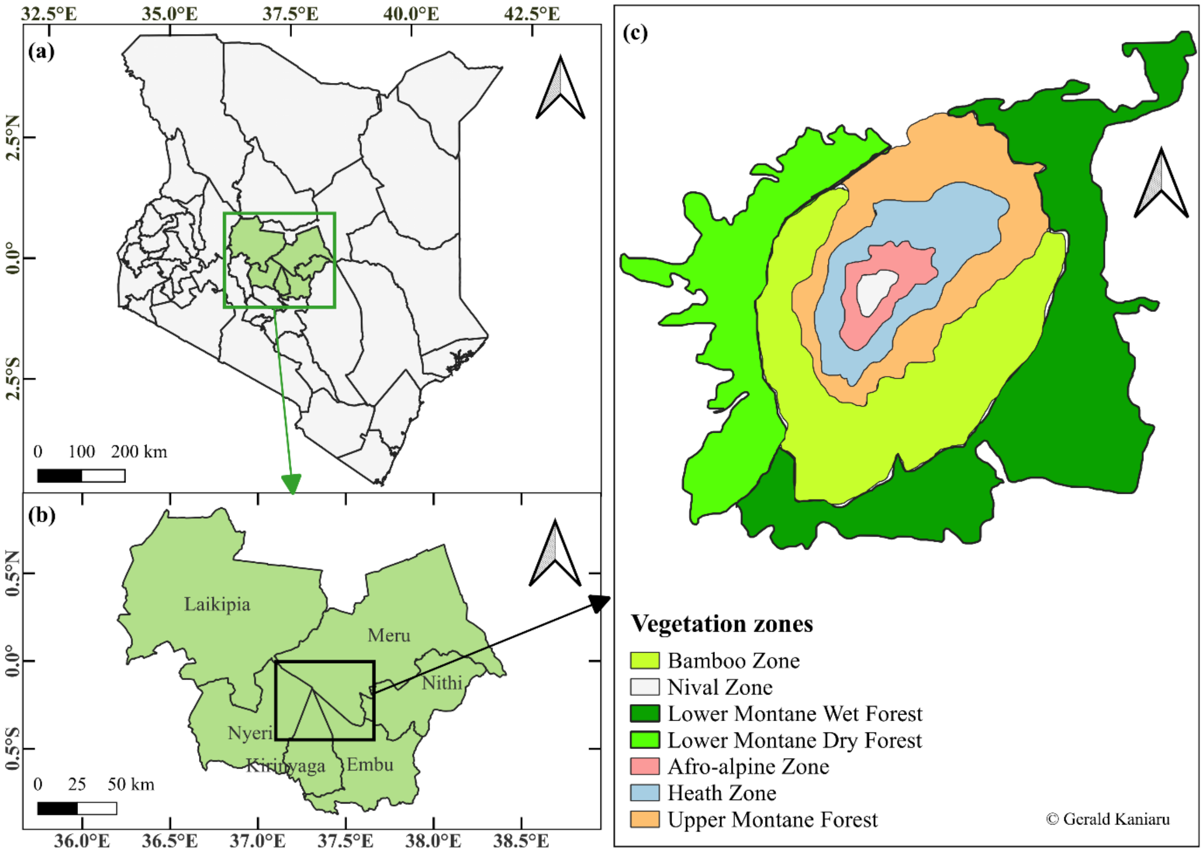

Figure 3.1. (a) Map of Kenya showing the Six Counties (b) Intersection of the Six Counties Occupied by Mt. Kenya; (c) Vegetation Zones of Mt. Kenya

3.1.2. Geography

Mount Kenya, a stratovolcano considered extinct, is part of the larger Mount Kenya National Park, a UNESCO World Heritage Site (UNESCO, 1997). It has three main peaks: Batian (5,199 meters), Nelion (5,188 meters), and Lenana (4,985 meters). The mountain's high elevation and cool climate support several glaciers, although climate change has significantly reduced their extent. Notable ice formations on the mountain include the Lewis Glacier, Tyndall Glacier, and Diamond Glacier.

Numerous rivers originate from Mount Kenya's glaciers and melting snowfields, with the most significant being the westward-flowing Ewaso Ng'iro. This river provides water to surrounding ecosystems and communities. Additionally, the mountain's slopes feature several small lakes, including Lake Alice, Lake Ellis, and Lake Michaelson.

3.1.3. Precipitation

Precipitation on Mount Kenya exhibits diverse patterns influenced by its high elevation and proximity to the equator. The mountain experiences varying forms of precipitation at different altitudes, resulting in a range of climates and ecosystems. The lower slopes of Mount Kenya, characterized by a mix of tropical and subtropical climates, receive abundant rainfall throughout the year. The convergence of northeast and southeast trade winds near the mountain brings moisture from the Indian Ocean, resulting in frequent showers and thunderstorms. Rainfall in this region averages between 1,000 and 2,500 millimeters annually.

As one ascends the mountain, the climate transitions from the lower slopes' lush forests to alpine and montane zones. The middle altitudes, ranging from approximately 2,500 to 3,500 meters, experience a wet season from March to May, followed by a drier season from June to September. During the wet season, precipitation primarily occurs as rainfall, while the drier season sees a mix of rainfall and snowfall. The average annual precipitation in this zone ranges from 500 to 1,500 millimeters. At higher elevations above 3,500 meters, Mount Kenya features an afro-alpine climate. This zone is characterized by lower temperatures, high winds, and a significant reduction in rainfall. Precipitation in the form of snow and hail is more common in these higher regions, especially during the colder months between October and February. The snowfall contributes to the formation of glaciers on the peaks of Mount Kenya, although their extent has been diminishing due to the impacts of climate change. The precipitation patterns can vary from year to year due to the influence of climate change and natural variability.

3.1.4. Humidity

Humidity patterns on Mount Kenya vary with altitude, prevailing weather systems, and the influence of local topography. Generally, humidity levels tend to decrease as one ascends higher on the mountain. The lower slopes of Mount Kenya often experience higher humidity levels. The proximity to the equator, combined with dense vegetation and forest cover, contributes to a moist environment. These areas can have moderate to high humidity, especially during the wetter seasons.

In the afro-alpine zone, humidity levels tend to be lower with drier and cooler air. The combination of colder temperatures and reduced moisture availability results in lower humidity. The lack of significant vegetation in the afro-alpine zone further contributes to the lower humidity levels experienced at these elevations. Humidity patterns can also be influenced by local weather systems and seasonal variations. The presence of rainfall, temperature fluctuations, and wind patterns can all impact humidity levels on Mount Kenya. Therefore, while a general trend of decreasing humidity with altitude can be observed, specific conditions can vary.

3.1.5. Temperature

Temperature patterns on Mount Kenya vary with altitude, latitude, and the influence of weather systems. The mountain exhibits distinct temperature zones, each characterized by unique climatic conditions. At the lower elevations of Mount Kenya, temperatures are generally warmer due to the proximity to the equator. The lower slopes range from the base up to approximately 2,500 meters. Daytime temperatures can range from mild to hot, with averages ranging from 20 to 30 °C. Nighttime temperatures are usually cooler, dropping to around 10 to 15 °C. As one ascends higher on the mountain, the temperature steadily decreases. At 2,500–3,500 metre elevation, daytime temperatures tend to range from 10 to 20 °C, while nighttime temperatures can drop to 5 °C or lower. In the afro-alpine zone, daytime temperatures often range from 0 to 10 °C, while nighttime temperatures can drop below freezing, especially during the colder months.

Weather systems and seasons also play a role in temperature variations on the mountain. During the wet season, which typically occurs from March to May and a shorter period from October to December, temperatures can be slightly cooler due to increased cloud cover and precipitation. The dry season, from June to September and January to February, tends to have more stable and warmer temperatures.

3.1.6. Vegetation

The Afromontane ecosystem is divided into distinct vegetation zones. The pictorial representation of these zones is demonstrated in Figure 3.2 below.

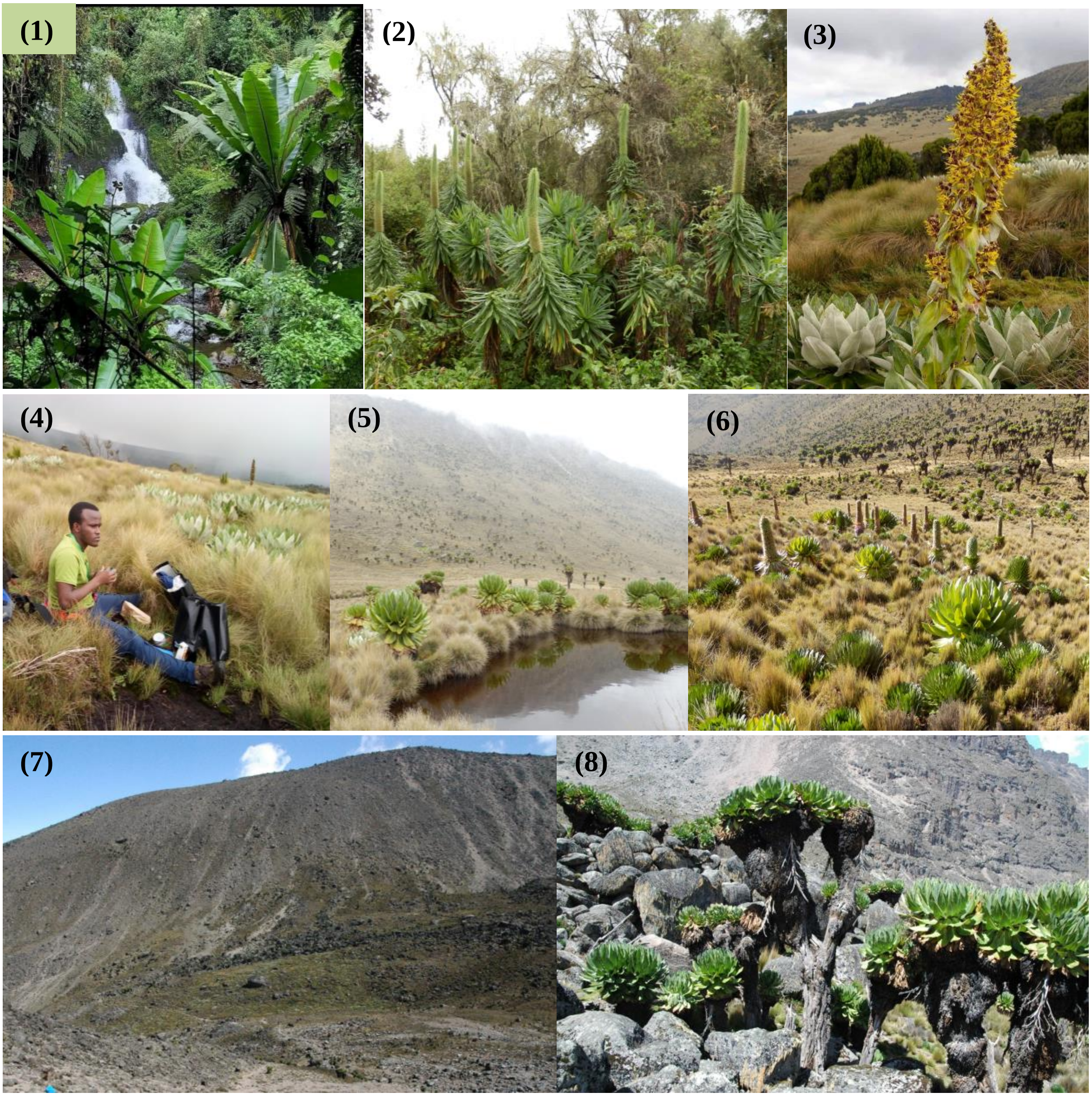

Figure 3.2: Pictorial Representation of the Vegetation Zones of Mount Kenya (1 & 2: Montane Zone, 3 & 4: Heath and Moorland Zone, 5 & 6: Upper Alpine Zone, 7 & 8: Nival Zone)

3.1.6.1. Montane Zone

This zone is dominated by the *Podocarpus latifolius-Hagenia abyssinica-Oldeania alpina* community. Other plant species in this zone include *Lobelia bambuseti* (endemic to Kenya only), *Alchemilla argyrophylla, Discopodium penninervium*, *Diclis bambuseti*, *Hypericum revolutum, Poa leptoclada, Kniphofia thomsonii, Rubus friesiorum, Agrostis kilimandscharica, Silene afromontana, Stellaria sennii, Polygonum afromontanum, Rubus keniensis, Trifolium burchellianum*, and *Impatiens hoehnelii* (endemic to Kenya only).

3.1.6.2. Heath and Moorland Zone

This zone is above the montane forest and lower alpine zones. It consists of ericaceous bushland and tussock moorland. The ericaceous zone includes *Erica arborea, Erica trimera, Helichrysum chionoides*, and *Haplocarpha rueppelii* species, among others. The moorland zone is made up of large herbs (*Dendrosenecio keniensis* and *Lobelia spp*.), gentians (*Swertia* spp.), and tussock sedges (*Carex monostachya* and *Festuca pilgeri*). Between the tussock sedges are *Geranium vagans, Alchemilla johnstonii*, and *Alchemilla abyssinica,* as well as small, creeping herbs such as *Lysimachia serpens,* among other species.

3.1.6.3. Upper Alpine Zone

This zone extends from 3800–4550 m a.s.l. It is dominated by giant rosettes of groundsels and Lobelias. They include *Dendrosenecio keniensis, Dendrosenecio keniodendron* (endemic to Mt. Kenya and dominating the steeper parts), *Lobelia deckenii* (endemic to the mountains of Tanzania), and *Lobelia gregoriana* (endemic to Kenya and Uganda). Other species in this zone include *Carduus keniensis* (endemic to both Mt. Kenya and the Aberdares), *Lobelia telekii*, *Agrostis trachyphylla, Arabis alpina, Carex monostachya, Carduus schimperi* subsp. *platyphyllus, Senecio keniophytum* (endemic to Mt. Kenya), *Gladiolus crassifolius, Haplocarpha rueppelii, Ranunculus oreophytus, Helichrysum spp*., and *Luzula abyssinica.*

3.1.6.4. Nival Zone

This zone extends from 4,500 to 5,199 metres. It is characterised by rocky and barren terrain with minimal vegetation cover. However, the little vegetation is composed of scattered giant groundsels, *Helichrysum spp., Lobelia spp., Arabis alpina, Senecio purtschelleri,* and *Valeriana kilimandscharica*.

3.2. Selection of *Arabis alpina* L. (Brassicaceae)

3.2.1. Morphological Characteristics and Adaptations of *Arabis alpina*

Arabis alpina L., also known as alpine rock cress, is a perennial pioneer herb in the family Brassicaceae. It creeps from a rootstock and has star-shaped hairs on all of its parts. It has slightly toothed obovate, oblanceolate, or oblong leaves. It has white flowers in a simple raceme with petals about 15mm long. Its fruits are irregular, purple, and winged, with pods up to 50 mm long. This species inhabits specific habitats that fulfil its ecological needs. Primarily, it is found in montane grasslands, rocky slopes (including cliffs, scree slopes, and talus fields), crevice habitats, and subalpine zones. These habitats offer the essential conditions for the plant to flourish, overcoming the distinctive challenges presented by the Afromontane environment.

Figure 3.3: Morphological Characteristics of *Arabis alpina*

This species has developed specific adaptation strategies to thrive in its environment. Its leaves are arranged in a circular pattern at the base, conserving heat and minimizing water loss by reducing the exposed surface area. Its deep root system allows it to access deep soil water, enabling seedling survival during drought and water scarcity. The extensive roots stabilize the plant on rocky substrates, preventing it from being dislodged by strong winds. The compact growth habits and low stature of *A. alpina* conserve energy and resources by minimizing the surface area exposed to harsh climatic conditions.

The species' small leaves reduce water loss through transpiration and protect against desiccation in the dry and windy conditions of the Afromontane ecosystem. Additionally, the species possesses fine, dense hairs on leaves and stems, creating a moisture-trapping microclimate that minimizes water loss and protects against excessive solar radiation and wind. These morphological adaptations enable the species to withstand the extreme climatic conditions within its ecosystem.

3.2.2. Ecological Characteristics of *Arabis alpina*

This species actively participates in nutrient cycling through photosynthesis, absorbing carbon dioxide from the atmosphere and contributing to the carbon cycle. As it eventually decomposes, the nutrients it contains are released back into the soil, enriching it and facilitating nutrient cycling. This species thrives in rocky slopes, crevices, and subalpine zones, contributing to the formation and stability of plant communities in these harsh environments. This colonization creates favourable conditions for other plant species to thrive, enhancing overall biodiversity and bolstering ecosystem resilience. Furthermore, *A. alpina*'s deep root system plays a crucial role in stabilizing the soil, particularly on rocky substrates, by securely anchoring the plant and preventing soil erosion.

3.2.3. *Arabis alpina* as a Model Species

This species is a distant cousin of the model species *Arabidopsis thaliana* (L.) Heynh. (Couvreur *et al*., 2010). It has a mixed mating system, with both selfing and outcrossing individuals (Tedder *et al*., 2011). However, the outcrossing rates in *A. alpina* are significantly low, suggesting a pronounced tendency for self-fertilization (Buehler *et al*., 2012). The species is diploid, reproducing sexually through seed production and asexually via stoloniferous growth (Buehler *et al*., 2012). Its genetic and reproductive variability contribute to its adaptability to changing climatic conditions.

Arabis alpina has been utilized as a model species to study the evolution of life histories, adaptive evolution, and the phylogeography of Italian mountains (Koch *et al*., 2006; Ansell *et al*., 2008). Consequently, it was selected as an ideal candidate for investigating plant species' responses to fluctuating climatic conditions due to its use as an evolutionary and ecological model species (Buehler *et al*., 2012). This species holds ecological significance, exhibits a wide distribution across elevation gradients, and displays diverse reproductive characteristics.

3.3. Sampling Design

The belt transect method was used. The pathway from the 3000m starting point to the 4800m end point was used as the transect line. This transect line was marked at 100-meter intervals, and the area between each interval was referred to as a 'block'. In each block, 5m x 5m plots were randomly placed, and the identified plots were sampled. Twenty-eight (28) sample plots of *A. alpina* were identified and assessed within an altitude range of 3,763m to 4,600m.

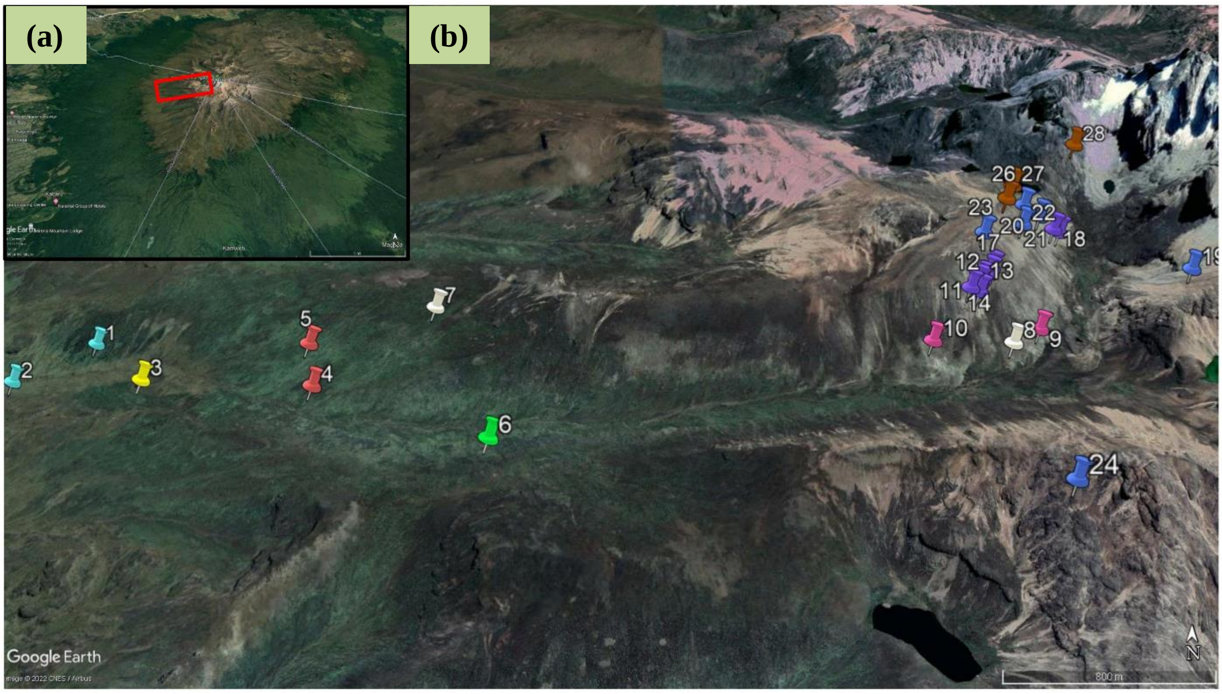

Figure 3.4: (a) Mt. Kenya Forest Reserve with the sampled area highlighted in a red text box; (b) Distribution of sample plots of *Arabis alpina* across the elevation gradient

Table 1: Legends of Sample Plots of *Arabis alpina*

Alt. range	Block Number	Latitude	Longitude	Labels of samples
3700-3800	1	-0.16688	37.2967	📌
3800-3900	2	-0.16608	37.2981	📌
3900-4000	3	-0.16693	37.2929	📌
4000-4100	4	-0.16449	37.2955	📌
4100-4200	5	-0.16435	37.2951	📌
4200-4300	6	-0.16407	37.2956	📌
4300-4400	7	-0.16393	37.2957	📌
4400-4500	8	-0.15951	37.2986	📌
4500-4600	9	-0.15918	37.2978	📌

3.4. Data Collection

3.4.1. Assessing the Onset and Duration of Flowering in *Arabis alpina*

The plants were visually inspected daily to detect the first flower. The date of the first flower opening was recorded for each observed plant. Visual inspection continued during the entire flowering period. The date of the final flower withering and fruit development onset were recorded for each plant. Diligent and consistent monitoring ensured complete coverage of the flowering period.

3.4.2. Seed Collection of *Arabis alpina*

The racemes of *A. alpina* individuals were bagged with a mesh bag to prevent seed loss during wind dispersal. Their pods were then hand-picked at their optimum stage of development (point of natural dispersal) from individuals tied in mesh bags.

3.4.3. Determination of Seed Mass and Seed Number in *Arabis alpina*

The number of *A. alpina* seeds was counted individually for each plot, and the averages were calculated per plot. The seeds were then placed in seed containers, sealed, and labelled with a unique seed collection number. In the seed laboratory, an analytical weighing balance was utilized to measure the mass of each seed accession collected from every plot, and subsequently, the average seed mass per plot was calculated.

3.4.4. Germination Trials and Survival Scoring of *Arabis alpina* Seedlings

Fifty (50) seeds were randomly selected from each seed accession and divided into five (5) replicates, each containing ten (10) seeds. The seeds from each replicate were planted in pots filled with soil samples collected from the specific plots where the seed collection was obtained. The sown seeds were then placed in a germination incubator.

The temperature was initially set at 11.5°C from 9:00 a.m. to 12:00 p.m. and gradually adjusted to 4°C from 12:01 p.m. to 7:00 p.m. Additionally, the relative humidity was set at 35% at midmorning (10:00 a.m.) and subsequently adjusted at intervals up to 90% at 7:00 p.m. These conditions replicated the temperature and humidity fluctuations experienced in the study area (UNEP-WCMC, 2022).

Regular monitoring of the germination pots was conducted. The pots were rearranged to prevent positional variation, and the seeds were watered to maintain moist soil, facilitating effective germination. Germination scoring commenced when the plumules attained a minimum length of 2 mm and persisted until the number of detected shoots stabilized.

The number of germinated seeds was counted and recorded, and the percentage germination rates of the sown *A. alpina* seeds were subsequently calculated using the formula below:

$$\textbf{Germination rate (\%)} = \frac{\textbf{Number of germinated } \textit{A. alpina} \textbf{ seeds}}{\textbf{Total number of sown seeds}} \textbf{ x 100}$$

After germination, the seedlings were continuously monitored for their survival and growth. The species' seedling survival rate was regularly assessed by recording the number of seedlings that remained healthy and actively growing. Any instances of seedling mortality or signs of stress or damage were carefully noted. The number of surviving seedlings was recorded at each monitoring interval. The percentage seedling survival rates of the sown *A. alpina* seeds were calculated using the formula below:

$$\textbf{Seedling Survival rate (\%)} = \frac{\textbf{Number of surviving } \textit{A. alpina} \textbf{ seedlings}}{\textbf{Total number of sown seeds}} \textbf{ x 100}$$

3.5. Soil Sampling and Analysis

Ten soil sub-samples were randomly collected from each plot at a depth of 15 cm. The sub-samples were mixed to form a composite sample for each plot. The composite samples were then analyzed for soil characteristics such as pH, electrical conductivity, carbon, nitrogen, and phosphorus.

3.5.1. Soil pH

1) The soil samples were spread out and dried. A 2 mm sieve was used to sieve the soil samples, and the sieved soil sections were collected for examination.
2) Duplicate samples of the composite soil (6 grams each) were prepared for each plot.
3) One set of soil samples received 15 mL of distilled water, while the other set received 15 mL of 0.01 M $CaCl_2$.
4) The samples were shaken for 30 minutes using a mechanical shaker and left to settle for another 30 minutes.
5) The pH of the soil samples was determined using a pH meter and glass electrodes.

3.5.2. Soil Electrical Conductivity (EC)

1) A small amount of reagent-grade KCl was dried in an oven at 60 °C for 2 hours, chilled in a desiccator, and stored in a tightly sealed bottle.

2) 0.7456 g of potassium chloride (KCl) salts were dissolved in DI water, transferred to a 1-litre flask, thoroughly mixed, and brought to volume.
3) Air-dried soil (50 g) was weighed, put into a 100-mL glass beaker, and mixed with DI water (50 mL) using a glass rod and allowed to stand for 30 minutes.
4) The suspension was further swirled, and the clear filtrate was transferred into a 50-mL bottle. A conductivity cell was immersed in the solution, and the reading was taken.

3.5.3. Soil Carbon (C)

1) The Walkley-Black method was used to oxidise the organic carbon in the soil (FAO, 2020).
2) The soil samples were sieved with a 2-mm sieve to remove the coarse soil particles.
3) The sieved portion of the soil sample was further ground with a 0.5-mm sieve.
4) The sieved soil samples were weighed to 1 g and placed in a conical flask.
5) 10 mL of 1N potassium dichromate was pipetted and stirred.
6) In a measuring cylinder, 20 mL of concentrated (36N) sulfuric acid was measured and poured into the soil-potassium dichromate mixture. Bubbles and considerable heat were produced. The mixture was allowed to cool for 20 minutes.
7) 5 mL of 85% orthophosphoric acid (H_3PO_4) and 5.0 mL of diphenylamine sulphonate indicator were added to the soil-potassium dichromate-concentrated sulfuric acid mixture.
8) The volume was raised to 200 mL by adding distilled water. The phosphoric acid ensured that a good endpoint was obtained when titrating with ferrous sulphate.
9) The mixture was titrated with 0.5 N ferrous sulphate.
10) 10 mL of potassium dichromate was titrated in a blank without soil.
11) Changes in the colour of the mixture were noticed from dark blue to green and finally to a pale green colour as the endpoint was approached.
12) One drop endpoint was obtained and the percentage carbon was calculated using the formulas below:

$$\text{Normality of Ferrous Sulphate} = \frac{10}{\text{Volume of the blank}}$$

$$\text{\% Oxidizable Carbon} = \frac{(\text{Volume.blank-Volume.sample}) \times 0.3 \times \text{Ferrous Sulphate Normality}}{\text{Weight of air-dry soil}}$$

$$\text{\% Total organic carbon} = \frac{100}{77} \times \text{Oxidizable Carbon}$$

3.5.4. Soil Nitrogen (N)

1) One gram of air-dried soil sample was weighed and placed in a 250-mL digestion flask, followed by one (1) gram of mixed catalyst and eight (8) mL of concentrated sulfuric acid.
2) The mixture was shaken and then placed in a Khejdahl digestion block at a low temperature of 120 °C for one hour.
3) The temperature was raised to 330 °C and continued to be heated until the solution turned colourless. The solution was allowed to cool.
4) Distilled water (25 mL) was added to the solution and well mixed until there was no further dissolution of the sediment.
5) The contents were allowed to cool and were topped up with water to 50 mL.
6) The mixture was allowed to settle, and the clear solution was extracted from the upper part of the tube for further analysis.
7) An aliquot of 10 mL of the clear solution was placed into a Kjeldahl distillation flask and fitted into the distillation system.
8) In the 250-mL conical flask, 10 mL of 40% sodium hydroxide were quickly added and distilled into the 2% H3BO3, which contained four drops of the mixed indicator.
9) The contents were titrated with H2SO4 at 0.01 N, changing their colour from green to pink. The equations used to calculate the percentage of nitrogen are as follows:

$$NH_3 + H_3BO_3 \rightleftarrows NH_4 + H_2\ BO_3$$

$$NH4^{+}+H_2BO_3\text{-}+H_2SO_4 \rightleftarrows NH_4HSO_4+H_3BO_3$$

$$\%\ \text{Nitrogen} = \frac{\text{Normality.acid x 14 x Titre x vol. extracted}}{\text{Weight.sample x 1000 x extracted aliquot (mL)}} \text{ x 100}$$

3.5.5. Soil Phosphorus (P)

1) The calorimetric method of determining the amount of phosphorus in the soil was used (Okalebo *et al*., 1993).
2) Reagents were prepared for soil sample analysis. They include double acid, reagent A, reagent B, standard P stock solution, and secondary P standard solution.
3) To prepare Double Acid, fifteen (15) litres of distilled water, 14 mL of concentrated H_2S0_4, and 83 mL of conc. HCI were mixed in a 20-litre bottle.
4) The volume was brought to 20 litres by adding distilled water.
5) To prepare Reagent A, 12g of $(NH4)_2MoO_4$ was dissolved in distilled water (250 mL). 0.2908g of $KSbOC_4HO_4$ was dissolved in 100 mL of distilled water.

6) $5NH_2S0_4$ was prepared by dissolving concentrated H_2S0_4 (148 mL) in 1000 mL of distilled water. The three solutions were combined in a volumetric flask and brought to volume by adding distilled water up to 2 litres.
7) To prepare Reagent B, 1.056 g of ascorbic acid was dissolved in every 200 mL of Reagent A (O'Halloran & Cade-Menun, 2007).
8) To prepare a standard P stock solution, 0.4393 g of KH_2PO_4 was weighed into a 1-litre volumetric flask. Then, 500 mL of distilled water was poured into the flask, and the solution was shaken until the salt dissolved. The volume was further diluted with distilled water. Five drops of C_7H_8 were added to eliminate microbial activity.
9) For the preparation of a secondary P standard solution, 5 mL of the P stock solution with a P concentration of 100 ppm was added to a 100 mL volumetric flask using a pipette. Distilled water was immediately added to reach the desired volume.
10) A soil sample weighing 5 g was placed in a 100-mL conical flask, and 50 mL of double acid was added to it using a pipette.
11) This was repeated for all the samples collected in each of the sampled plots. The conical flask was tightly sealed and shaken vigorously for 30 minutes.
12) Filtration of the soil samples was performed using filter papers, and the filtrate was collected in beakers for further analysis.
13) 0 mL, 1 mL, 2 mL, 3 mL, 4 mL, and 5 mL of secondary standard solution with a P concentration of 5 ppm were pipetted into 50 mL volumetric flasks to prepare the standard P solutions.
14) 5 mL of double acid were added to each of the flasks, followed by 20 mL of distilled water.
15) 8 mL of reagent B was added to each flask, followed by distilled water to make it to volume and thoroughly mixed.
16) Readings were taken using a spectrophotometer after 15 minutes, during which the mixture was allowed to stand.
17) A calibration curve was used to determine the phosphorus concentration in the soil samples.
18) A one-mL aliquot of the mixture was pipetted into a 50-mL conical flask, followed by 25 mL of distilled water and 8 mL of reagent B.
19) Distilled water was added to the mixture and mixed thoroughly. The readings were taken after 15 minutes of allowing the mixture to stand.

3.6. Data Exploration and Statistical Analysis

All statistical analyses were performed using R version 4.3.1 (R Core Team, 2023).

3.6.1. Scatter Plots

These plots were generated using the 'ggpubr' R package (Kassambara, 2023) to demonstrate the trend in the soil characteristics of the sample plots of *A. alpina*, illustrate the relationship between the reproductive fitness traits of *A. alpina* and elevation, and examine trade-offs among these fitness traits.

3.6.2. Multiple Pairwise Comparison Box Plots

These plots were used to visualize the specific vegetation zone(s) that contributed to the overall variation in the reproductive fitness traits of *A. alpina*. They were generated using the 'ggpubr' (Kassambara, 2023) and 'ggplot2' (Wickham, 2009) packages. These plots provide a visual representation of the results obtained from the ANOVA analysis.

3.6.3. One-Way Analysis of Variance (ANOVA)

This analysis assessed the variations in reproductive fitness traits of *A. alpina* across the lower alpine, upper alpine, and nival zones. These vegetation zones were treated as independent variables, while the fitness traits were considered dependent variables. The residuals versus fitted values plots were used to assess the homogeneity of variances, whereas the normality plots of residuals were used to check for normality in this analysis. These diagnostic plots are demonstrated in Appendices 1–2. Any outliers detected in these plots that could have affected normality and homogeneity of variances were removed to satisfy the test assumptions.

3.6.4. Linear Mixed-Effects Models (LMMs)

This statistical analysis was employed using the 'lmer' function in the lme4' package (Bates *et al*., 2015) to determine variations in reproductive fitness traits of *A. alpina* along the elevation gradient and the degree of association between these traits. The analysis accounted for confounding factors such as soil physicochemical properties and spatial autocorrelation originating from different vegetation zones, blocks within these zones, and plots within the blocks. Spatial autocorrelation was taken into account in this analysis since certain sample plots of *A. alpina* demonstrated similarities in species composition, physical factors, and environmental conditions due to their close proximity. The variables utilized and the structure of this analysis are summarized in Table 2.

Table 2: Summary of Linear Mixed-Effects Models Showing Fixed Variables, Nested Random Effects, and Excluded Variables for Model Convergence

Research Questions	Fixed effects	Response variable	Random effects	Excluded random factors
The impact of elevation on the onset and duration of flowering in *A. alpina*	Elevation	Onset of flowering	Soil properties; Vegetation zone/Block/Plot	Block/Plot
		Duration of flowering	Soil properties; Vegetation zone/Block/Plot	Vegetation zone/Block/Plot
Trade-offs between the onset and duration of flowering in *A. alpina* along the elevation gradient	Onset of flowering	Duration of flowering	Elevation; Soil properties; Vegetation zone/Block/Plot	Vegetation zone/Block/Plot
The effects of elevation on the seed mass and seed number of *A. alpina*	Elevation	Seed mass and Seed number	Vegetation zone/Block/Plot	Plot
Trade-offs between seed mass and seed number of *A. alpina* along the elevation gradient	Seed mass	Seed number	Elevation; Soil properties; Vegetation zone/Block/Plot	Plot
The effects of elevation on the germination and seedling survival rate of *A. alpina*	Elevation	Germination and Seedling Survival	Soil properties; Vegetation zone/Block/Plot	Block/Plot
Trade-offs between germination and seedling survival rates of *A. alpina* along elevation	Germination	Seedling Survival	Elevation; Soil properties; Vegetation zone/Block/Plot	Plot
The effects of seed mass and seed number on the germination and seedling survival rates of *A. alpina* along elevation	Seed mass and Seed number	Germination and Seedling survival	Soil properties; Vegetation zone/Block/Plot	Vegetation zone/Block/Plot

3.6.5. Model Convergence

This analysis was used to evaluate the performance and accuracy of the models in estimating the response variables, specifically the reproductive fitness traits of *A. alpina* in this case. Residuals versus fitted values plots and Q-Q plots of standardized residuals were generated for each model using the 'plot()' function in the 'ggpubr' package (Kassambara, 2023), and the identified outliers were removed. These diagnostic plots are demonstrated in Appendices 3–6. In models that failed to converge, nested random factors with low variances on the response variable were excluded to ensure convergence.

3.6.6. Principal Component Analysis (PCA)

This statistical analysis transformed a set of correlated variables into a smaller set of uncorrelated variables known as principal components. The principal components were visualized using the 'biplot()' function in the 'factoextra' package (Kassambara & Mundt, 2020). *Arabis alpina* individuals were grouped into vegetation zones using the concentration ellipses in the PCA biplots. The 'ggsci' package (Xiao, 2023) was used to apply the 'igv' color palette to the concentration ellipses in order to differentiate the vegetation zones. These biplots provide valuable insights into the interrelationships among the reproductive fitness traits of *A. alpina*, soil physicochemical parameters, and elevation.

CHAPTER FOUR: RESULTS

4.1. Soil Characteristic Variations Across the Elevation Gradient

The physicochemical properties of the soil demonstrate a declining trend, whereas the soil electrical conductivity shows an increasing trend across the elevation gradient. These trends are graphically illustrated in Figure 4.1.

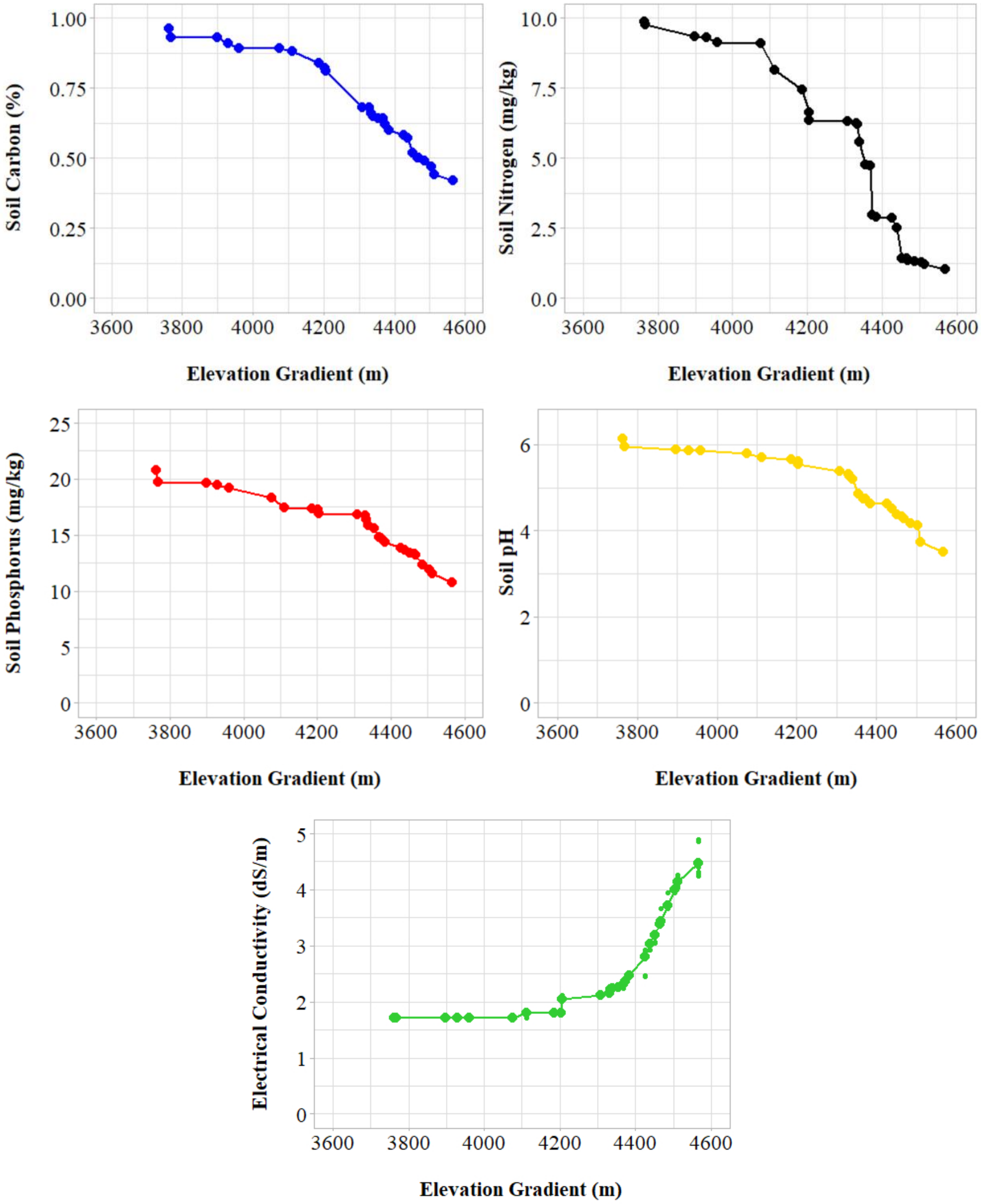

Figure 4.1: Trends in Soil Physicochemical Variables along the Elevation Gradient

4.2. Direction and Magnitude of *Arabis alpina* Reproductive Fitness Traits Along the Elevation Gradient

Significant variations were observed in the reproductive fitness traits of *A. alpina* across different vegetation zones, as depicted in Figure 4.2 below.

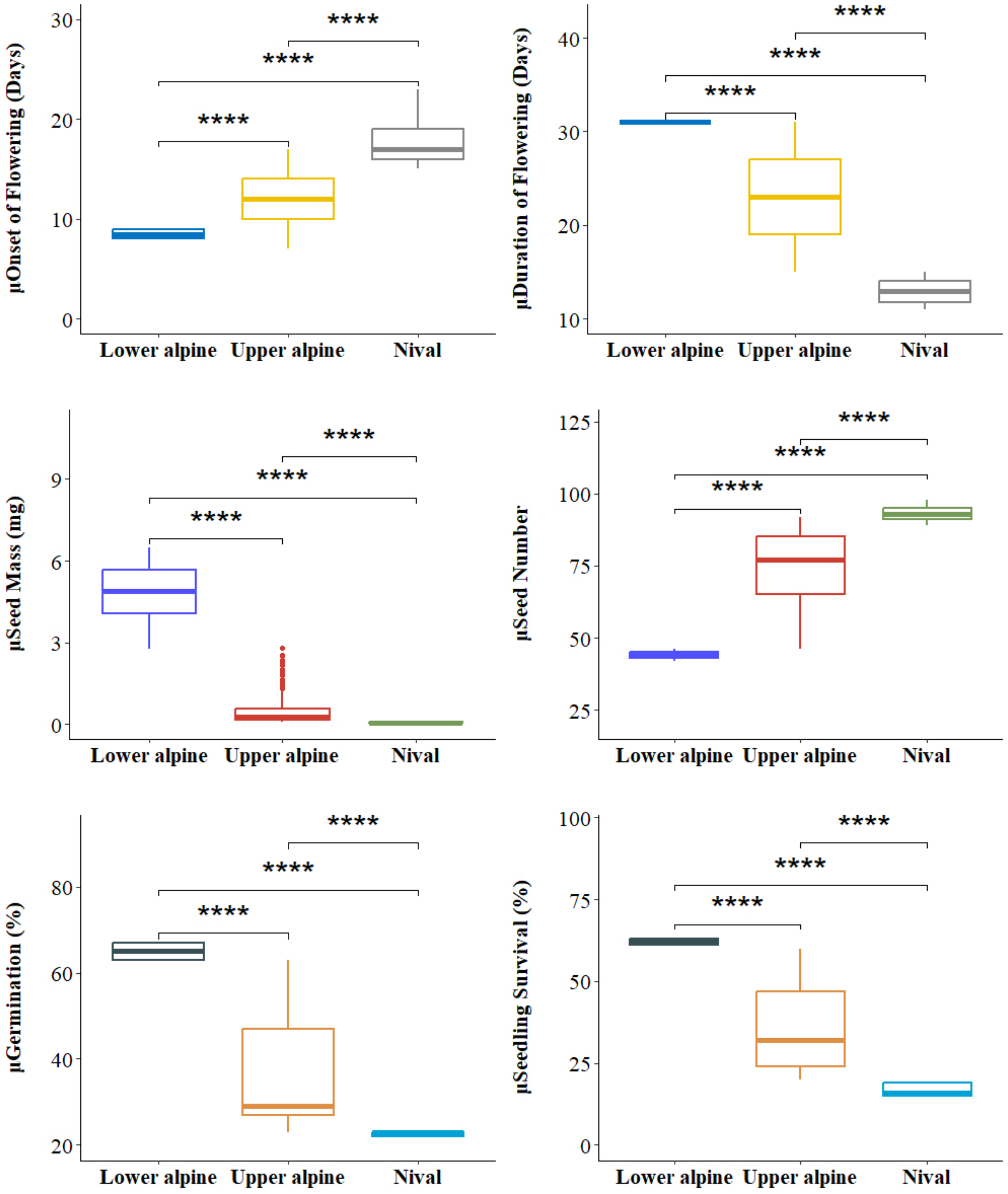

Figure 4.2: Variation in the Reproductive Fitness Traits of *Arabis alpina* Across the Sampled Vegetation Zones

These traits show variable responses to elevation changes. The onset of flowering and seed number in *A. alpina* have a linearly increasing relationship with elevation. Conversely, the species' duration of flowering, seed mass, germination, and survival rates have a linearly decreasing relationship with elevation, as shown in Figure 4.3 below.

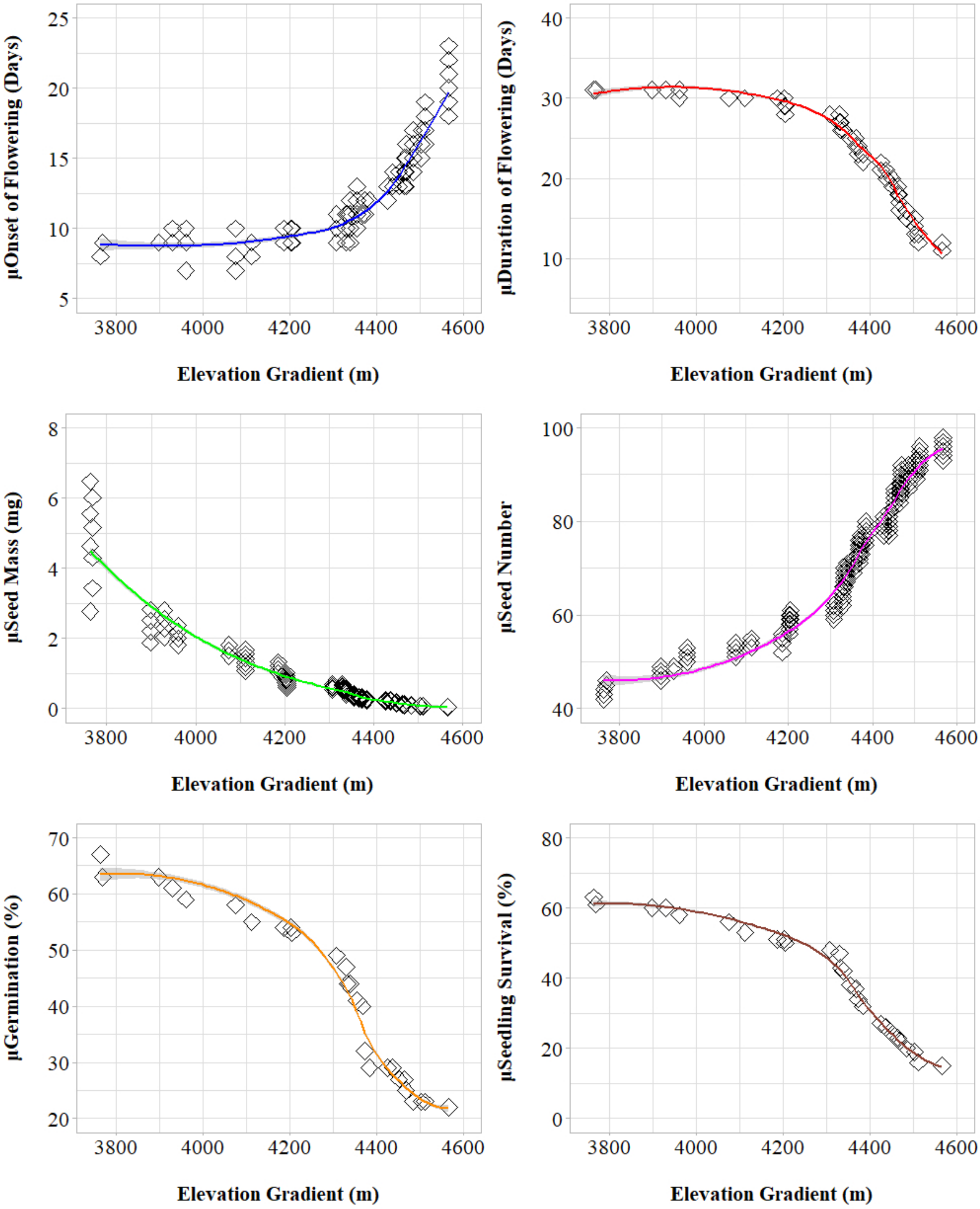

Figure 4.3: Linear Relationships between the Fitness Traits of *Arabis alpina* and Elevation Gradient

The onset of flowering in *A. alpina* and elevation are positively correlated (Table 3, p. 32), with an effect size of 0.008 (Model Equation 1). This implies that for every 100-meter elevation rise, there is a delay of 0.8 days (≡19 hours and 20 minutes) in the species' onset of flowering.

Onset of flowering (days) = 0.008 (Elevation) – 21.14 ***(Model Equation 1)***

In contrast, there is a negative correlation between the species' duration of flowering and elevation (Table 3, p. 32), with an effect size of 0.017 as shown in Model Equation 2. This indicates that for every 100 meters of elevation gain, the species' duration of flowering decreases by 1.7 days. (≡1 day and 17 hours).

Duration of flowering (days) = 98.82 – 0.017 (Elevation) ***(Model Equation 2)***

The seed mass of *A. alpina* and elevation are negatively correlated (Table 3, p. 32), with an effect magnitude of 0.004 as demonstrated in Model Equation 3. This indicates that for every 100-meter increase in elevation, the species' average seed mass decreases by 0.4 mg.

Seed mass (mg) = 18.87 − 0.004 (Elevation) ***(Model Equation 3)***

The seed number of *A. alpina* and elevation are positively correlated (Table 3, p. 32), with an effect size of 0.07 as illustrated in Model Equation 4. This implies that for every 100-meter increase in elevation, the species' average seed number increases by 7 seeds.

Seed number = 0.07 (Elevation) − 220.9 ***(Model Equation 4)***

The germination rate of *A. alpina* and elevation are negatively correlated (Table 3, p. 32), with an effect size of 0.08 as presented in Model Equation 5. This implies that for every 100-meter increase in elevation, there is an 8% decrease in the species' average germination rate.

Germination (%) = 372.74 − 0.08 (Elevation) ***(Model Equation 5)***

Similarly, the species’ seedling survival rate and elevation are negatively correlated (Table 3, p. 32), with an effect size of 0.08, as demonstrated in Model Equation 6. This indicates that the species' seedling survival rate decreases by 8% for every 100 meters of elevation gain.

` Seedling Survival (%) =378.16 – 0.08 (Elevation) ***(Model Equation 6)***

4.3. Trade-offs between the Reproductive Fitness Traits of *Arabis alpina*

In *A. alpina*, phenological and seed characteristics exhibit a declining linear correlation. Conversely, germination and seedling survival of the species portray an ascending linear association, as depicted in Figure 4.4 below.

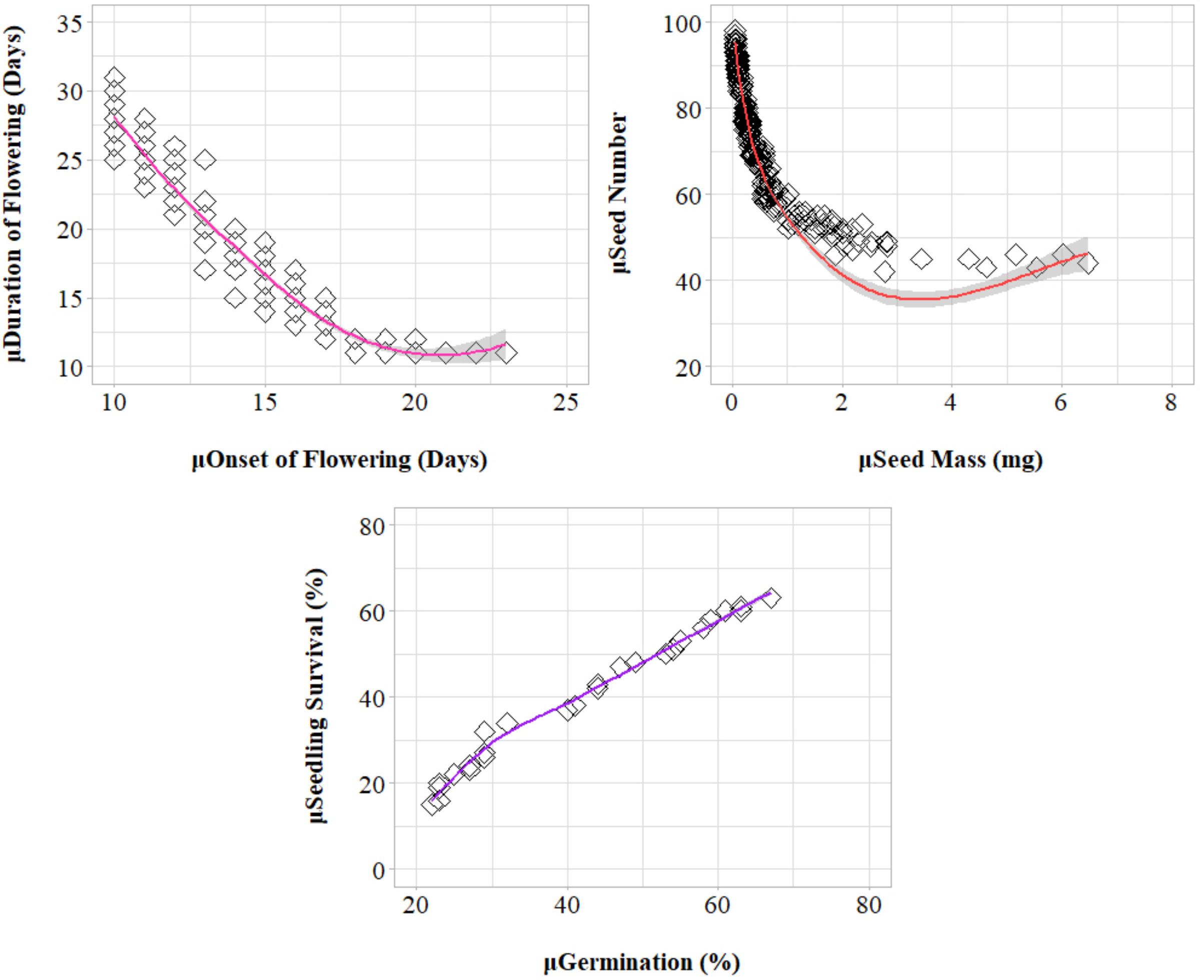

Figure 4.4: Linear Relationships between *Arabis alpina* Reproductive Fitness Traits

The onset and duration of flowering in *A. alpina* are negatively associated (Table 3, p. 32), with an effect size of 0.07, as displayed in Model Equation 7. This implies that a one-day delay in the species' onset of flowering leads to a reduction in its duration of flowering by 0.07 days (≡1 hour and 41 minutes), and vice versa.

Duration of flowering (days) = 24.16 – 0.07 (Onset of flowering) ***(Model Equation 7)***

The seed mass and seed number of *A. alpina* are negatively correlated (Table 3, p. 32), with an effect size of 8.25, as portrayed in Model Equation 8. This indicates that for every 1 mg decrease in the species' seed mass, there is a corresponding increase of 8 seeds, and vice versa.

Seed number = 81.00 – 8.25 (Seed mass) ***(Model Equation 8)***

The germination and seedling survival rates of *A. alpina* are positively correlated (Table 3, p. 32), with an effect size of 0.82, as depicted in Model Equation 9. This demonstrates that a 1% increase in the species' germination rate corresponds to a 0.82% increase in its seedling survival rate.

Seedling Survival (%) = 0.82 (% Germination) + 4.92 ***(Model Equation 9)***

4.4. Selection of Seed Traits and their Influence on the Fitness of *Arabis alpina*

A linearly increasing relationship between seed mass of *A. alpina* and its germination and seedling survival rates was observed. However, a linearly decreasing relationship between the seed number of *A. alpina* and its germination and survival rates was also observed as illustrated in Figure 4.5 below.

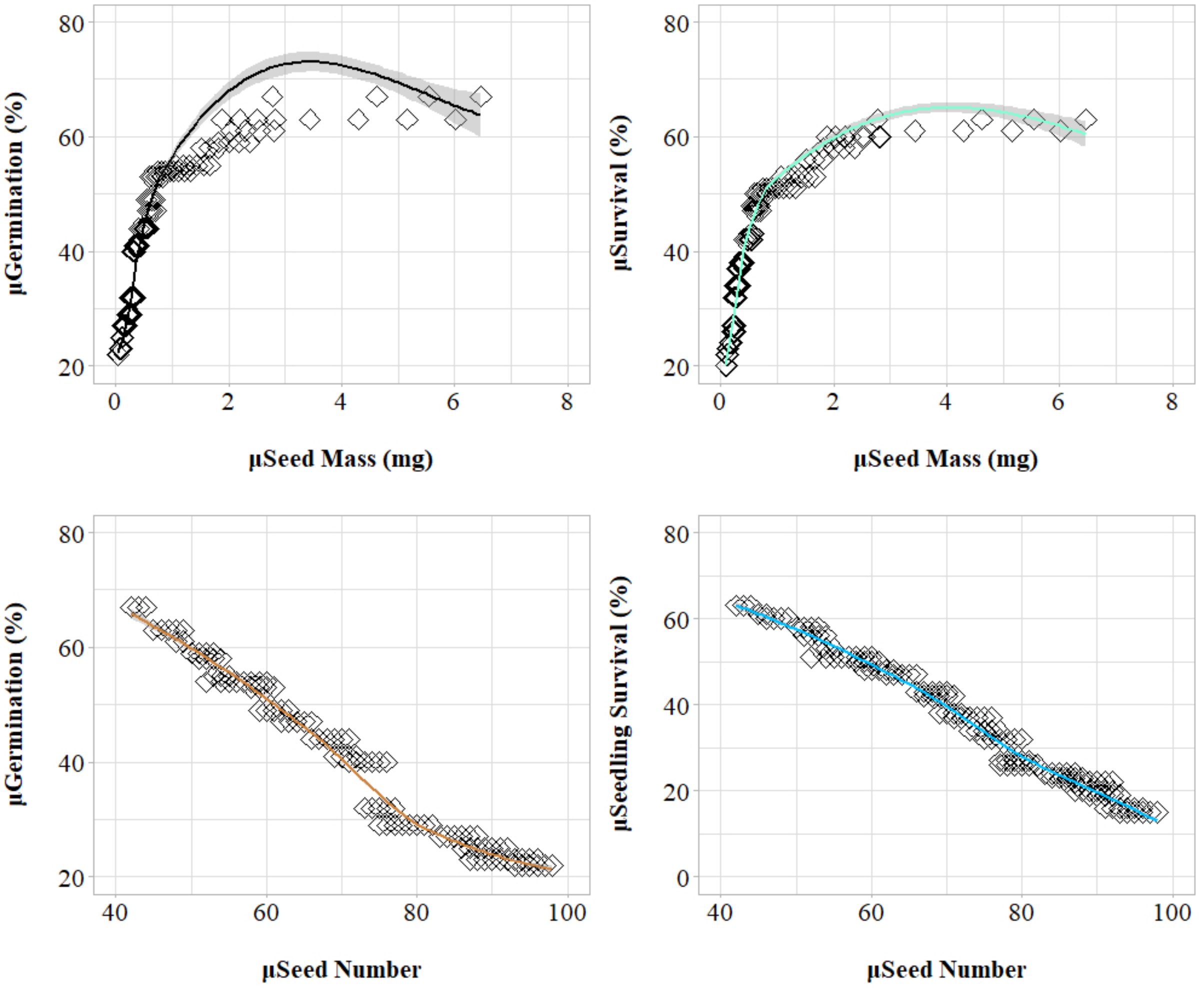

Figure 4.5: Linear Relationships between the Seed Traits of *Arabis alpina* and its Germination and Seedling Survival Rates

The seed mass and germination rate of *A. alpina* are positively correlated (Table 3, p. 32), with an effect size of 9.1 as indicated in Model Equation 10. This denotes that a 1 mg increase in the species' seed mass corresponds to a 9.1% decrease in its germination rate.

Germination (%) = 9.1 (Seed mass) + 29.1 ***(Model Equation 10)***

Similarly, the seed mass and seedling survival rate of *A. alpina* are positively correlated (Table 3, p. 32), with an effect size of 8.5 as shown in Model Equation 11. This entails that a 1 mg increase in the species' seed mass results in an 8.5% decrease in its survival rate.

Seedling survival (%) = 8.5 (Seed mass) + 26.61 ***(Model Equation 11)***

The seed number and germination rate of *A. alpina* are negatively correlated (Table 3, p. 32), with an effect size of 0.88 as shown in Model Equation 12. This indicates that the species' germination rate decreases by 0.88% for every increase in seed number by one seed.

Germination (%) = 102.42 − 0.88 (Seed number) ***(Model Equation 12)***

The species' seed number and its seedling survival rate are negatively correlated (Table 3, p. 32), with an effect size of 0.78 as demonstrated in Model Equation 13. This implies that for every increase in the species' seed number by one seed, there is a corresponding decrease of 0.78% in the species' seedling survival rate, and vice versa.

Seedling survival (%) = 93.57 − 0.78 (Seed number) ***(Model Equation 13)***

Table 3: Statistical Summary of the Inter-correlations among *Arabis alpina* Reproductive Fitness Traits, and Elevation

Predictor variable (x)	Outcome variable (y)	R^2_{adj}	RSE	F	df	P
Elevation	Onset of flowering	0.63	1.89	979.7	1, 579	<0.001
Elevation	Duration of flowering	0.74	3.14	1611	1, 579	<0.001
Elevation	Seed mass	0.81	0.32	2504	1, 579	<0.001
Elevation	Seed number	0.87	5.02	3936	1, 579	<0.001
Elevation	Germination	0.87	4.55	3814	1, 579	<0.001
Elevation	Seedling survival	0.86	5.04	3617	1, 579	<0.001
Onset of flowering	Duration of flowering	0.92	1.71	6787	1, 579	<0.001
Seed mass	Seed number	0.91	4.14	3022	1, 579	<0.001
Germination	Seedling survival	0.97	2.24	20710	1, 579	<0.001
Seed mass	Germination	0.59	7.98	848.9	1, 579	<0.001
Seed mass	Seedling survival	0.56	9.03	725.2	1, 579	<0.001
Seed number	Germination	0.96	2.49	14110	1, 579	<0.001
Seed number	Seedling survival	0.98	1.79	32820	1, 579	<0.001

4.5. Summary of Findings

A positive correlation between the onset of flowering in *A. alpina* and the elevation gradient was observed. This indicates that individuals at higher elevations exhibit an earlier onset of flowering, while their conspecifics at lower elevations exhibit a delayed onset of flowering. Conversely, there is a negative correlation between the duration of flowering in the species and the elevation gradient. A longer duration of flowering is associated with individuals at lower elevations, while a shorter duration of flowering is associated with individuals at higher elevations. Additionally, there is a negative correlation between the onset and duration of flowering in *A. alpina*. This entails that an earlier onset of flowering translates to a longer duration of flowering, while a delayed onset of flowering results in a shorter duration of flowering in *A. alpina*. These relationships are depicted in Figure 4.6.

The seed mass of *A. alpina* demonstrates a negative correlation with the increasing elevation gradient. Greater seed mass is strongly linked to individuals at lower elevations, while lesser seed mass is associated with individuals at higher elevations. On the other hand, the seed number of the species exhibits a positive correlation with increasing elevation. Higher seed numbers of *A. alpina* are associated with high-elevation habitats, while lower seed numbers are associated with low-elevation habitats. The seed mass and seed number of *A. alpina* are negatively correlated along the elevation gradient. This trade-off entails that in high elevations, the species reduces its seed mass at the expense of higher seed numbers, whereas in lower elevations, the species reduces its seed number at the expense of higher seed mass. These associations are illustrated in Figure 4.6.

The germination and survival rates of *A. alpina* and elevation are negatively correlated. Lower-elevation individuals of *A. alpina* exhibit higher germination and survival rates, while their conspecifics at higher elevations exhibit lower germination and survival rates. Additionally, the germination rate of the species positively correlates with its survival rate. This implies that an increase in the germination rate of *A. alpina* results in an increase in its seedling survival rate, and vice versa. These relationships are depicted in Figure 4.6.

A higher seed mass is positively associated with increased germination and survival rates of *A. alpina*, whereas a lower seed mass is positively associated with decreased germination and seedling survival rates of the species. A higher seed number of *A. alpina* is positively correlated with decreased germination and survival rates, whereas a lower seed number is positively correlated with higher germination and seedling survival rates.

The selection of single-seed provisioning and mass seed production as resource allocation strategies depends on the elevation gradient and soil characteristics. These associations are illustrated in Figure 4.5 below. Lower elevations possess sufficient levels of soil carbon (C), nitrogen (N), and phosphorus (P), a higher pH, and low electrical conductivity (EC). In contrast, higher elevations possess limited amounts of soil carbon, nitrogen, and phosphorus, a lower pH, and higher electrical conductivity. The species responds to the limited amounts of soil nutrients, a lower pH, and a higher electrical conductivity by prolonging its onset of flowering, shortening its duration of flowering, reducing its seed mass, increasing its seed number, and decreasing its germination and survival rates. These associations are illustrated in Figure 4.6.

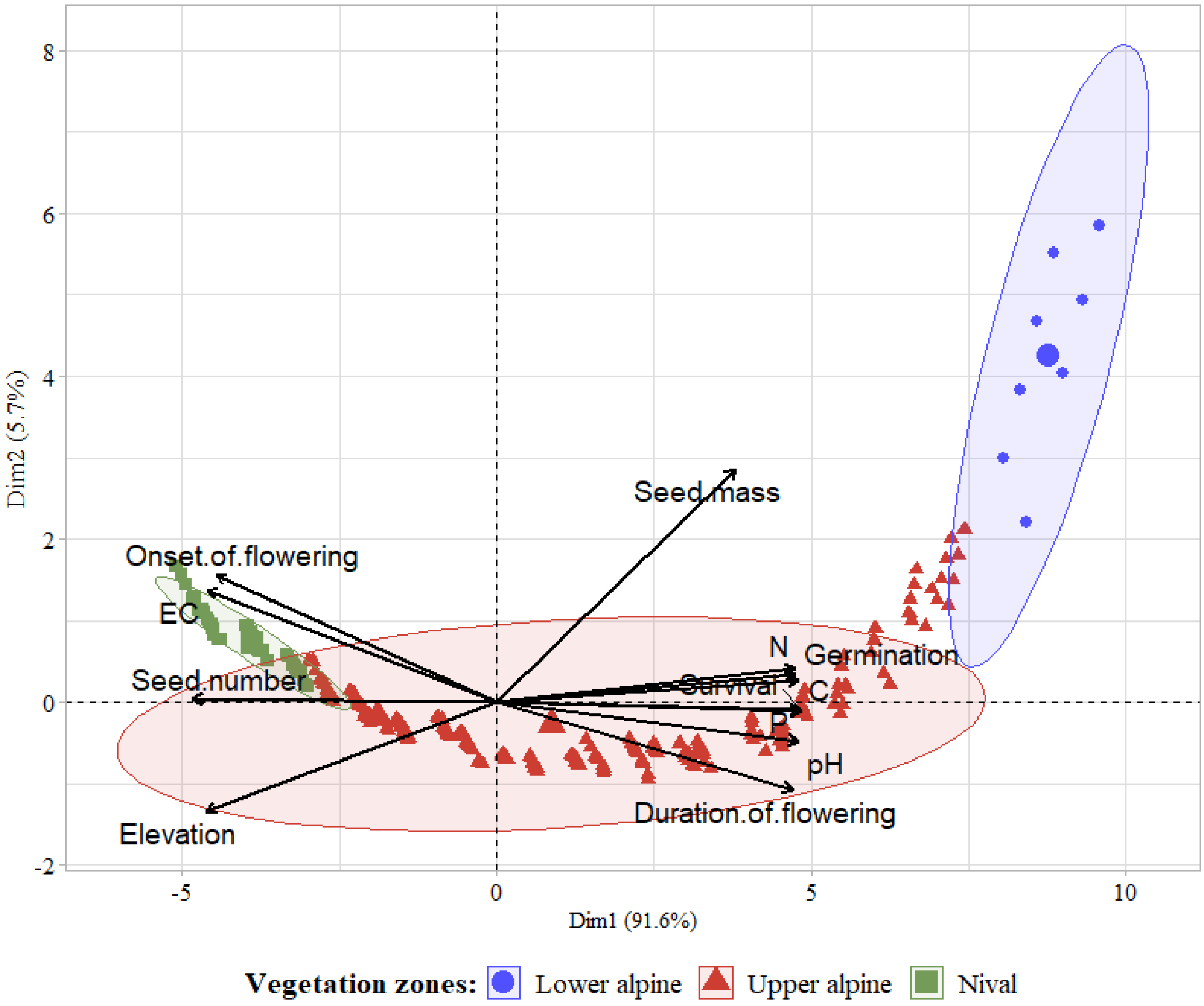

Figure 4.6: Multivariate Associations among the Reproductive Fitness Traits of *Arabis alpina*, Soil Characteristics, and Elevation

CHAPTER FIVE: DISCUSSION, CONCLUSION, AND RECOMMENDATIONS

5.1. Discussion

5.1.1. Adaptive Responses of *Arabis alpina* Fitness Traits to Elevation Change

5.1.1.1. Effects of Elevation on Onset and Duration of Flowering in *Arabis alpina*

Phenological shifts occur in response to changing climatic cues (Crimmins *et al*., 2011). With increasing elevation, *A. alpina* exhibits a delayed onset and shorter duration of flowering, while at lower elevations, the species experiences an early onset and prolonged duration of flowering. This can be attributed to elevation-dependent environmental conditions, including temperature, wind intensity, frost events, UV radiation, precipitation patterns, and soil characteristics such as nutrient availability, pH levels, and water retention capacity. Consequently, *A. alpina* adjusts its reproductive timing to synchronize with favourable environmental conditions, ensuring successful reproduction.

5.1.1.2. Effects of Elevation on Seed Traits of *Arabis alpina*

Seed mass and seed number of *A. alpina* display phenotypic plasticity in response to elevation changes. At higher elevations, *A. alpina* individuals produce more, lighter seeds compared to their lower-elevation conspecifics, which produce fewer, heavier seeds. This finding is supported by the energy constraint hypothesis (Baker, 1972; Zhang *et al*., 2004) and aligns with studies on seed trait selection in *Ipomoea purpurea* (Mojonnier, 1998) and seed mass variation in *Banksia marginata* (Vaughton & Ramsey, 2001).

5.1.1.3. Effects of Elevation on Germination and Survival Rates of *Arabis alpina*

Germination and seedling survival rates of *A. alpina* vary across the elevation gradient. According to the results of the linear mixed effects model and the PCA analysis, there is a relationship among elevation, soil nutrients, germination, and survival rates of *A. alpina*. Higher germination and survival rates of *A. alpina* are strongly correlated with nutrient-rich soils at lower elevations, while lower germination and survival rates of the species are highly associated with nutrient-limited soils at higher elevations. The low survival rates of *A. alpina* can be attributed to lower temperatures, which lead to frost events during the species' growing season. These frost events can harm the delicate seedlings of the species, thereby impeding their growth and establishment (Larcher *et al*., 2010; Neuner, 2014). Furthermore, the exposure of its seeds to low temperatures may also hamper their survival in the soil (Jaganathan *et al*., 2020).

5.1.2. Adaptive Responses of *Arabis alpina* Fitness Traits to Soil Property Variations

5.1.2.1. Flowering Responses of *Arabis alpina* to Soil Property Variations

The results of the linear mixed effects model and PCA analysis demonstrate that the observed variations in the timing of onset and duration of flowering in *A. alpina* can be attributed to changes in soil carbon concentration. These findings align with the results of a study on the impact of plant phenology on soil resource acquisition (Nord & Lynch, 2009). Organic matter decomposition releases nitrogen and phosphorus, which stimulate vigorous vegetative growth in *A. alpina* (Hu *et al*., 2021). This provides energy for an earlier onset and prolonged duration of flowering in lower-elevation individuals through photosynthesis (Wang *et al*., 2009; Vayssières *et al*., 2020; Sergeeva *et al*., 2021). Limited soil carbon, nitrogen, and phosphorus in high-elevation soils may have delayed the species' onset of flowering until sufficient nutrient levels are available (Müller *et al*., 2017; Mwende Muindi, 2019). Additionally, the species may have shortened its duration of flowering in response to nutrient limitations (Steffen & Kadereit, 2014).

Soil pH affects nutrient availability by influencing the solubility, mobility, and availability of nutrients for plant uptake (Neina, 2019; Penn & Camberato, 2019; Ferrarezi *et al*., 2022). An optimal pH of 6 in lower elevations promotes nutrient availability (Lavoie & Bradley, 2003), resulting in an earlier onset and longer duration of flowering. Conversely, the highly acidic pH of 3.5 at higher elevations limits nutrient availability (Mishra & Ramakrishnan, 1983; Lavoie & Bradley, 2003), causing a delayed onset of flowering in *A. alpina* until soil nutrients become accessible. Additionally, high-elevation *A. alpina* individuals exhibit a shorter duration of flowering in response to limited nutrient availability caused by acidic pH levels in high-elevation soils.

Soil electrical conductivity is a measure of the soil’s ability to conduct an electrical current (Seifi & Alimardani, 2010). The results of this study demonstrate that the variation in the timing of the onset and duration of flowering in *A. alpina* can also be attributed to changes in soil electrical conductivity along the elevation gradient. Increasing elevation correlates with higher electrical conductivity and salt content (Wilcox *et al*., 1957). This reduces soil water-holding capacity and impedes nutrient uptake by high-elevation *A. alpina* individuals (Ding *et al*., 2018; Silva *et al*., 2023). As a result, the species’ onset of flowering is delayed until the salt content decreases and water becomes available.

In addition, the species exhibits a shorter duration of flowering as a plastic response to higher salt content and limited water availability in their high-elevation habitats (Phillips *et al.*, 2011).

5.1.2.2. Responses of *Arabis alpina* Seed Traits to Soil Property Variations

Heavier *A. alpina* seeds can be attributed to higher nitrogen and phosphorus levels in lower-elevation soils, which enhance photosynthesis, nutrient uptake, and biomass accumulation (Baddeley *et al.*, 1994; Jiaying *et al.*, 2022). This finding is corroborated by a study on the effect of nitrogen addition on seed yield and yield components of *Leymus chinensis* in Songnen Plain, China (Chen *et al.*, 2013). In contrast, high-elevation individuals, growing in habitats with limited soil carbon, nitrogen, and phosphorus, yield seeds with reduced mass. This finding is corroborated by a study of the influence of elevation variations on the seed size of herbaceous plants in the Tatra Mountains of southern Poland (Olejniczak *et al.*, 2018).

Optimal pH levels ensure nutrient availability for seed development and the eventual production of heavier seeds in lower-elevation *A. alpina* individuals (Kołodziejek, 2017). Conversely, acidic pH limits nutrient availability at higher elevations (Mishra & Ramakrishnan, 1983; Lavoie & Bradley, 2003), hindering seed development and resulting in the production of lighter seeds (Kołodziejek, 2017). High conductivity at higher elevations hampers nutrient uptake and reduces soil water-holding capacity (Ding *et al.*, 2018; Silva *et al.*, 2023), resulting in reduced *A. alpina* seed mass.

5.1.2.3. Germination and Survival of *Arabis alpina* in Varied Soil Properties

In lower elevations, enhanced germination and seedling survival of *A. alpina* can be attributed to nitrogen and phosphorus availability (Boczulak *et al.*, 2014), as well as increased soil water holding capacity (He *et al.*, 2014). Nitrogen stimulates growth hormones such as auxins, cytokinins, gibberellins, and ethylene that promote root development, shoot development, seed germination, and responses to stress in *A. alpina*, respectively (Weiss & Ori, 2007; Wang & Irving, 2011; Tsukanova *et al.*, 2017; Sosnowski *et al.*, 2023). It also enhances photosynthesis efficiency, nutrient uptake, and assimilation, and strengthens the species' tissues against environmental stressors (Anas *et al.*, 2020). Phosphorus, a component of ATP, supports energy transfer and storage and promotes photosynthesis, root development, disease resistance, and stress tolerance in *A. alpina* (Bechtaoui *et al.*, 2021; Kayoumu *et al.*, 2023; Khan *et al.*, 2023). Consequently, increased survival rates of *A. alpina* seedlings can be attributed to nitrogen and phosphorus-rich lower-elevation soils.

The nutrient-rich soils at lower elevations influence both plant growth and root development (Razaq *et al*., 2017). These factors are crucial for efficiently absorbing nutrients and water resources. In contrast, *A. alpina* individuals at higher elevations struggle to establish extensive root systems due to limited phosphorus availability (Niu *et al*., 2013), thus lowering their survival rates. Improved soil water-holding capacity ensures moisture availability for seed imbibition, resulting in earlier and more rapid germination. It also prevents seedling desiccation, thereby increasing the species' survival rate. Conversely, limited soil carbon in high elevations limits soil nitrogen and phosphorus availability and reduces soil water-holding capacity, hampering the germination and survival of *A. alpina* seedlings.

The germination and survival rates of *A. alpina* are influenced by soil pH, which impacts nutrient availability (Neina, 2019; Penn & Camberato, 2019; Ferrarezi *et al*., 2022). An optimal pH of 6 promotes optimal nutrient uptake, facilitating rapid seed germination (Osuna *et al*., 2015; Zhang *et al*., 2020). This leads to the development of healthier, more robust *A. alpina* seedlings with increased survival rates. Conversely, an acidic pH at higher elevations limits nutrient availability (Mishra & Ramakrishnan, 1983; Lavoie & Bradley, 2003). This hinders fast germination and results in weak seedlings with low survival rates. Soil pH at higher elevations deviates from the optimal range, leading to nutrient deficiencies that negatively impact germination, seedling development, and survival in *A. alpina* (Offord *et al*., 2014; Gentili *et al*., 2018; Johan *et al*., 2021).

At higher elevations, decreased germination rates of *A. alpina* can be attributed to high soil electrical conductivity that induces salt-induced osmotic stress (Khatri & Rathore, 2022). This stress limits water and nutrient availability and uptake by the species' seeds. Salts in soils with high conductivity increase the osmotic potential of the soil solution, creating an osmotic gradient that inhibits water extraction by *A. alpina* from the soil (Hussain Shah *et al*., 2021; Khondoker *et al*., 2023; Salman *et al*., 2023). This impedes growth and ultimately reduces the survival rate of high-elevation *A. alpina* individuals.

5.1.3. Trade-offs between the Reproductive Fitness Traits of *Arabis alpina*

5.1.3.1. Trade-off between Onset and Duration of Flowering in *Arabis alpina*

This trade-off involves the timing and duration of the reproductive phase in *A. alpina*. Early flowering in lower-elevation individuals maximizes resource utilisation, enabling the completion of their life cycles before unfavourable biotic (predation, competition, invasive species, diseases, parasites, and herbivory) and abiotic (temperature extremes, drought, frost, and soil erosion) factors occur (Elzinga *et al*., 2007; Hoiss *et al*., 2015; Abdala-Roberts *et al*., 2016). However, early flowering at high elevations may expose *A. alpina* individuals to adverse weather conditions and limited resources, hindering reproductive success (Thomson, 2010; Iler *et al*., 2019; Dorji *et al*., 2020; Kudo, 2022). As a result, high-elevation individuals adapt by delaying flower emergence until suitable conditions arise in their harsh habitats. Similarly, there are trade-offs associated with a longer duration of flowering in *A. alpina*. This species flowers earlier and allocates sufficient resources in nutrient-rich lower-elevation habitats to maintain an extended flowering period (Zhao *et al*., 2020).

A longer flowering period enhances fitness in *A. alpina* by increasing the chances of attracting pollinators, resulting in a higher seed set and reproductive output (Zimmerman & Gross, 1984; Kehrberger & Holzschuh, 2019). This finding is supported by a study on the phenological overlap of interacting species under changing climatic conditions (Rafferty *et al*., 2013). In high-elevation individuals, flowering is delayed until conditions are suitable for flower emergence (Kazan & Lyons, 2016; Cho *et al*., 2017). The results of the PCA analysis demonstrate that limited soil resources in higher-elevation habitats result in a shorter duration of flowering in *A. alpina*. This ensures the successful completion of the species' life cycle in high-elevation habitats characterized by nutrient limitations, environmental stressors such as strong winds, intense sunlight, and UV radiation, as well as biotic factors such as predation, disease, and competition. These factors can potentially reduce the species’ overall fitness. The trade-off between the onset and duration of flowering in *A. alpina* represents a balance between reproduction timing and resource allocation influenced by soil characteristics.

5.1.3.2. Trade-off between Seed Mass and Seed Number of *Arabis alpina*

This trade-off represents the inverse relationship between the mass of individual *A. alpina* seeds and the total number of seeds produced. It is supported by studies on functional trade-offs and phylogenetic dispersion of seed traits in the Mountains of Southwest China (Chen *et al*., 2018) and seed traits in *Ipomoea purpurea* (Mojonnier, 1998).

This trade-off can be attributed to the effects of varying soil characteristics along the elevation gradient. Lower-elevation individuals allocate sufficient soil resources and energy towards single-seed provisioning, resulting in fewer but heavier *A. alpina* seeds compared to high-elevation conspecifics. This finding is corroborated by a study on the functional trade-offs between seed traits in the mountains of southwest China (Chen *et al*., 2018). This reduction in seed number may limit dispersal potential and colonization capacity (Chen *et al*., 2018). Conversely, high-elevation conspecifics allocate limited resources and energy towards mass seed production, leading to the production of many lighter *A. alpina* seeds. This enhances the production of a large number of offspring capable of colonizing fragmented, nutrient-deficient habitats through wind dispersal. This finding aligns with a study on seed size changes in mountain herbaceous plants with elevation (Olejniczak *et al*., 2018). Ultimately, this trade-off represents a balance between investing resources in individual seed success and maximizing the overall reproductive output of *A. alpina*.

The study's findings revealed that higher germination rates of *A. alpina* lead to increased survival rates for the species in lower-elevation habitats. Conversely, reduced germination rates are associated with decreased survival rates for the species. During the germination phase, the species' seedlings are vulnerable to environmental stresses such as drought, extreme temperatures, herbivory, and competition from other plants. A faster germination rate allows *A. alpina* seedlings to withstand and survive these challenges (Duncan *et al*., 2019; Lai *et al*., 2019). If germination is delayed, the emergence of the seedlings may be compromised, reducing their chances of survival (Yang *et al*., 2015; Bianchi *et al*., 2019).

Germination triggers the growth of the seedling's first root and shoot systems. These structures are essential in facilitating the uptake of nutrients, water, and light for growth and survival. Successful germination ensures that the seedling can access essential resources early on, promoting its chances of survival and growth to maturity (Fernández-Pascual *et al*., 2021). This study observed that high-elevation *A. alpina* individuals exhibit low germination and survival rates. This may have been a result of the development of dormancy traits to cope with limited resources and harsh environmental conditions (Schütz, 2002; Fernández-Pascual *et al*., 2021; Peng *et al*., 2021).

5.1.4. Impact of Seed Trait Selection on Germination and Survival of *Arabis alpina*

Germination and seedling survival in *A. alpina* can be attributed to the variation in seed mass of the species across the elevation gradient. This finding aligns with the results of a study on the effects of elevation and seed mass on the germinability of *Allium* species (Ge *et al.*, 2020). Seed mass determines the initial energy reserves for *A. alpina* seedling establishment. Seeds from lower-elevation individuals are heavier, containing abundant stored nutrients (carbohydrates, lipids, and proteins) that fuel seedling establishment and early growth. Conversely, conspecifics from high elevations have lighter seeds with limited reserves. This affects the development of a robust root system and the acquisition of essential soil nutrients (Wang *et al.*, 2016). This leads to delayed seedling emergence (Önemli, 2004), slow and incomplete germination (Follmer *et al.*, 2021), reduced seedling vigour (Rose & Raymond, 2020), stunted growth (Zong *et al.*, 2020), and increased susceptibility to diseases and pests (Chowdhury *et al.*, 2022). Ultimately, these factors contribute to a decrease in the species' overall fitness.

Intense competition for resources, including light, water, and nutrients, is prevalent in tropical Afromontane environments (Horák *et al.*, 2023). Highly-fit seedlings from higher *A. alpina* seed mass possess a competitive advantage over weaker seedlings from lower seed mass (Hendrix *et al.*, 1991; Moles & Westoby, 2004; Metz *et al.*, 2010; May *et al.*, 2013; Da Silva *et al.*, 2023). In high-elevation areas, *A. alpina* individuals produce numerous lighter seeds, giving rise to seedlings with thin and fragile stems that are susceptible to damage from wind, rain, and herbivory. Consequently, these seedlings are likely to experience higher mortality rates.

5.2. Conclusion

Arabis alpina exhibits plastic responses to changes in elevation and soil characteristics, which impact its reproductive fitness. The species adjusts the timing and duration of flowering in response to variations in elevation gradients and soil properties. Individuals of *A. alpina* at higher elevations experience delayed onset and shorter durations of flowering, while their conspecifics at lower elevations exhibit earlier onset and prolonged durations of flowering. This adaptive strategy allows *A. alpina* to synchronize its reproductive timing with favourable environmental conditions, ensuring successful reproduction. At higher elevations, the species produces more and lighter seeds, while lower elevations yield fewer and heavier seeds. This trade-off reflects resource allocation and affects dispersal potential and colonization capacity. Additionally, *A. alpina* faces difficulties in seedling emergence, growth, and survival in high-elevation habitats due to nutrient limitations, lower temperatures, decreased oxygen levels, and exposure to intense sunlight and UV radiation. These factors result in delayed emergence, incomplete germination, reduced vigour, stunted growth, nutrient deficiencies, and increased vulnerability to diseases and pests, ultimately decreasing fitness.

Variations in soil characteristics play a crucial role in shaping the phenology, seed traits, germination, and seedling survival of *A. alpina*. Optimal soil conditions at lower elevations promote earlier onset and longer durations of flowering, heavier seed production, improved germination, and higher seedling survival rates. In contrast, nutrient limitations, acidic pH levels, and higher electrical conductivity at higher elevations delay flowering, reduce seed mass, hamper germination, and compromise seedling survival in *A. alpina*.

These plastic responses and trade-offs in reproductive fitness traits highlight the adaptive strategies employed by *A. alpina* to thrive in diverse ecological niches. This provides valuable insights into the ecological and evolutionary dynamics of *A. alpina* and sheds light on its ability to persist and adapt to varying habitats. This knowledge will aid in predicting the future distribution and population dynamics of this species and others in similar environments. By identifying the underlying mechanisms of phenotypic plasticity and adaptive responses to environmental changes, conservation strategies can be developed to safeguard biodiversity and protect vulnerable species amidst ongoing climate change.

5.3. Recommendations

1) **Conservation implications**: The findings of this study should be used to inform conservation strategies, including identifying priority areas for conservation and implementing habitat management practises to preserve the adaptive potential of *Arabis alpina* populations.
2) **Long-term monitoring**: To understand the long-term implications of phenotypic plasticity, long-term monitoring of *Arabis alpina* populations along the elevation gradient and across different soil types is recommended. This will allow researchers to observe population dynamics, assess the stability of phenotypic plasticity over time, and evaluate the fitness consequences of different phenotypic traits.
3) **Reproductive fitness in a broader context**: While this study evaluates specific aspects of reproductive fitness, such as flowering time, seed traits, germination, and seedling survival, other facets like pollination efficiency and seed dispersal are not explicitly examined. A more comprehensive understanding of the adaptive strategies employed by *Arabis alpina* can be achieved by incorporating a broader perspective on reproductive fitness.
4) **Expand the study to other species and ecosystems**: Similar investigations can be conducted on other plant species in different ecosystems, providing comparative data on phenotypic plasticity and contributing to a broader understanding of how plants adapt to environmental gradients. Expanding the study to include other ecosystems will enhance our knowledge of the general principles and patterns of phenotypic plasticity in response to environmental variation.

By acknowledging these recommendations, the findings of this study provide opportunities for further investigations, ensuring a comprehensive understanding of the complex interactions between environmental factors and the reproductive fitness traits of Afromontane plant species in the tropical Afromontane ecosystem.

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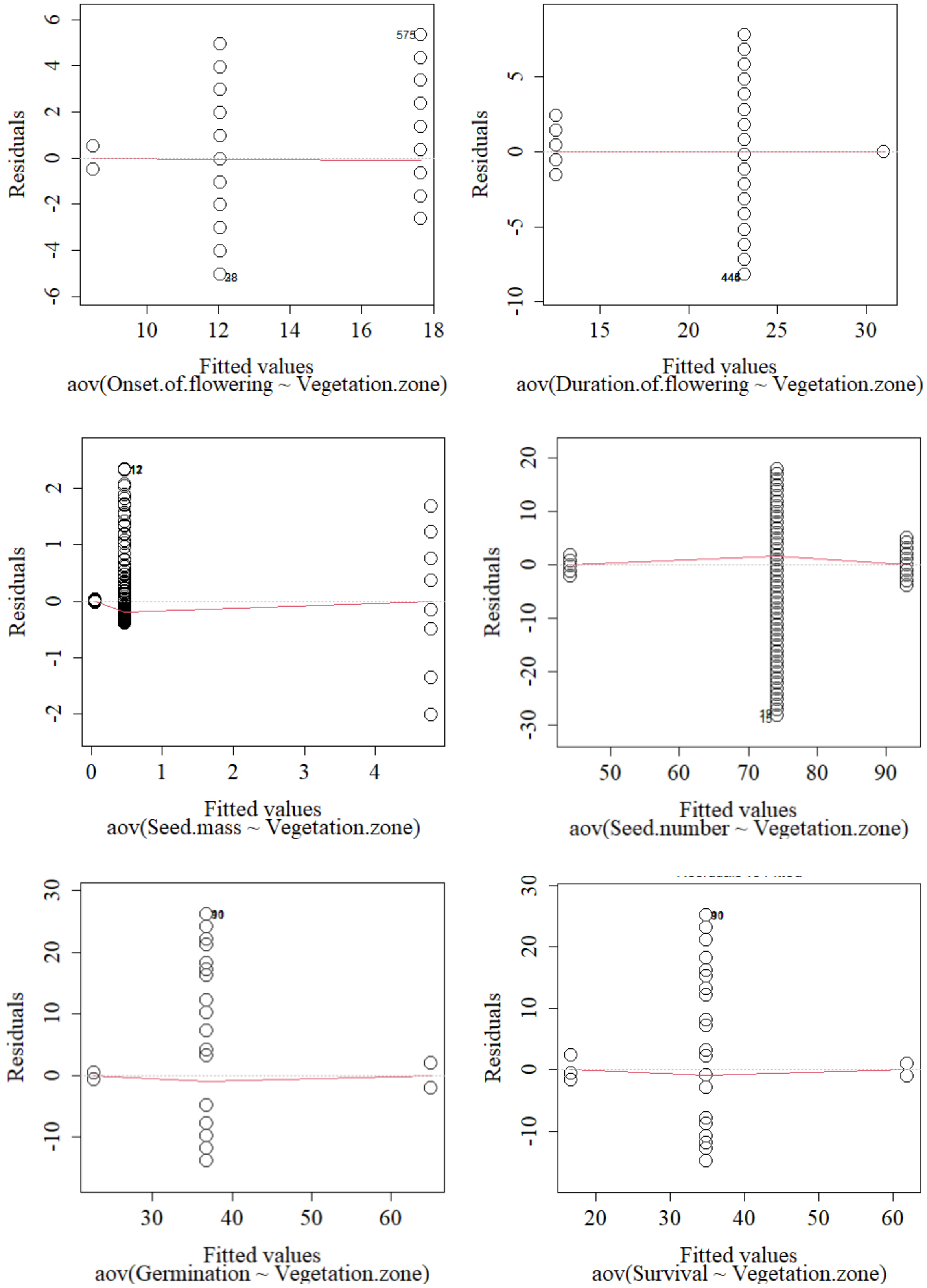
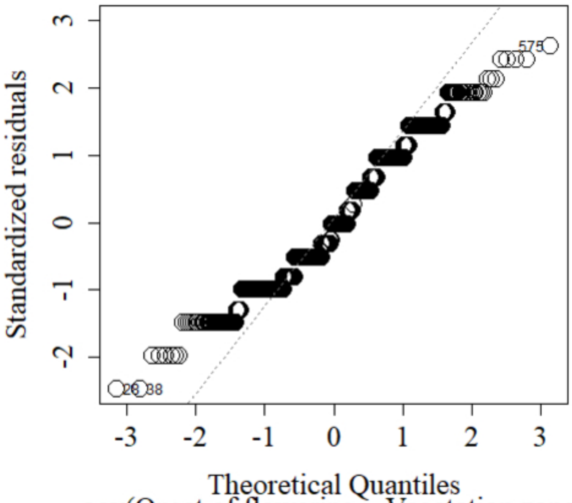
APPENDICES

Appendix 1: Residuals versus Fitted Values Plots Illustrating the Homogeneity of Variance in *Arabis alpina* Fitness Traits Across the Vegetation Zones



Appendix 2: Q-Q Plots of Standardized Residuals Illustrating Normality in the Fitness Traits of *Arabis alpina* Across the Vegetation Zones



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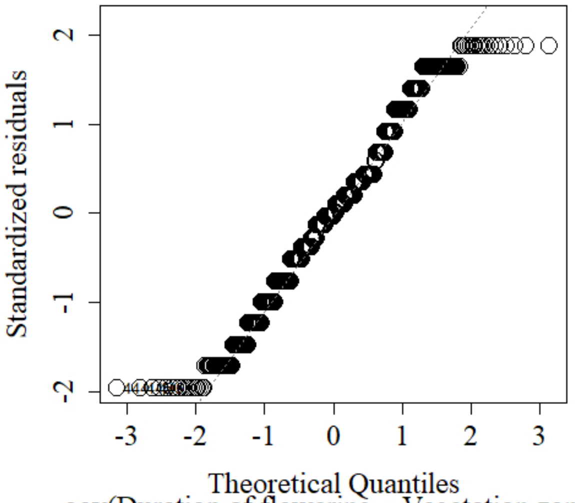

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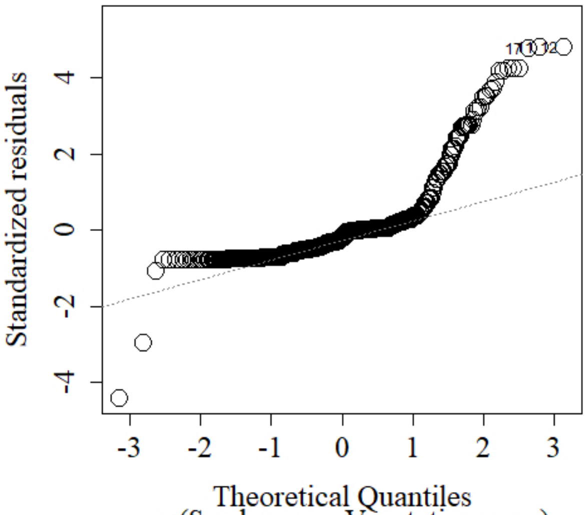
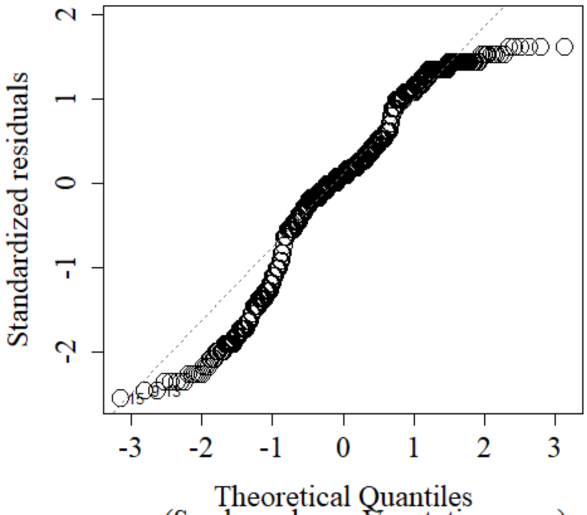


aov(Seed.mass ~ Vegetation.zone)



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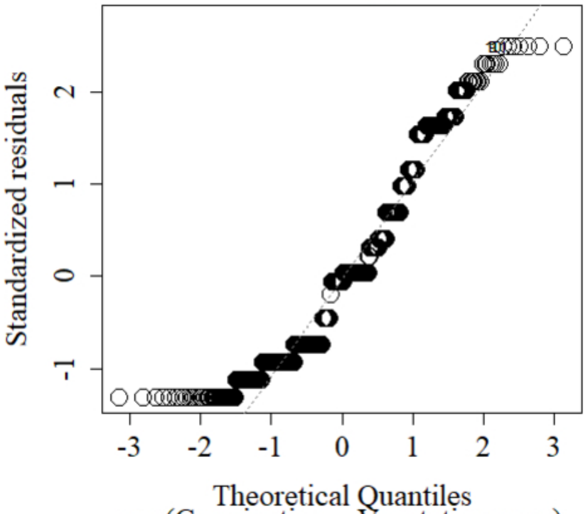
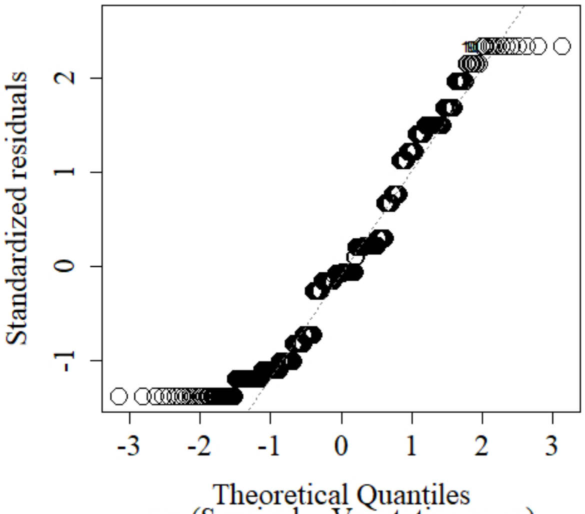
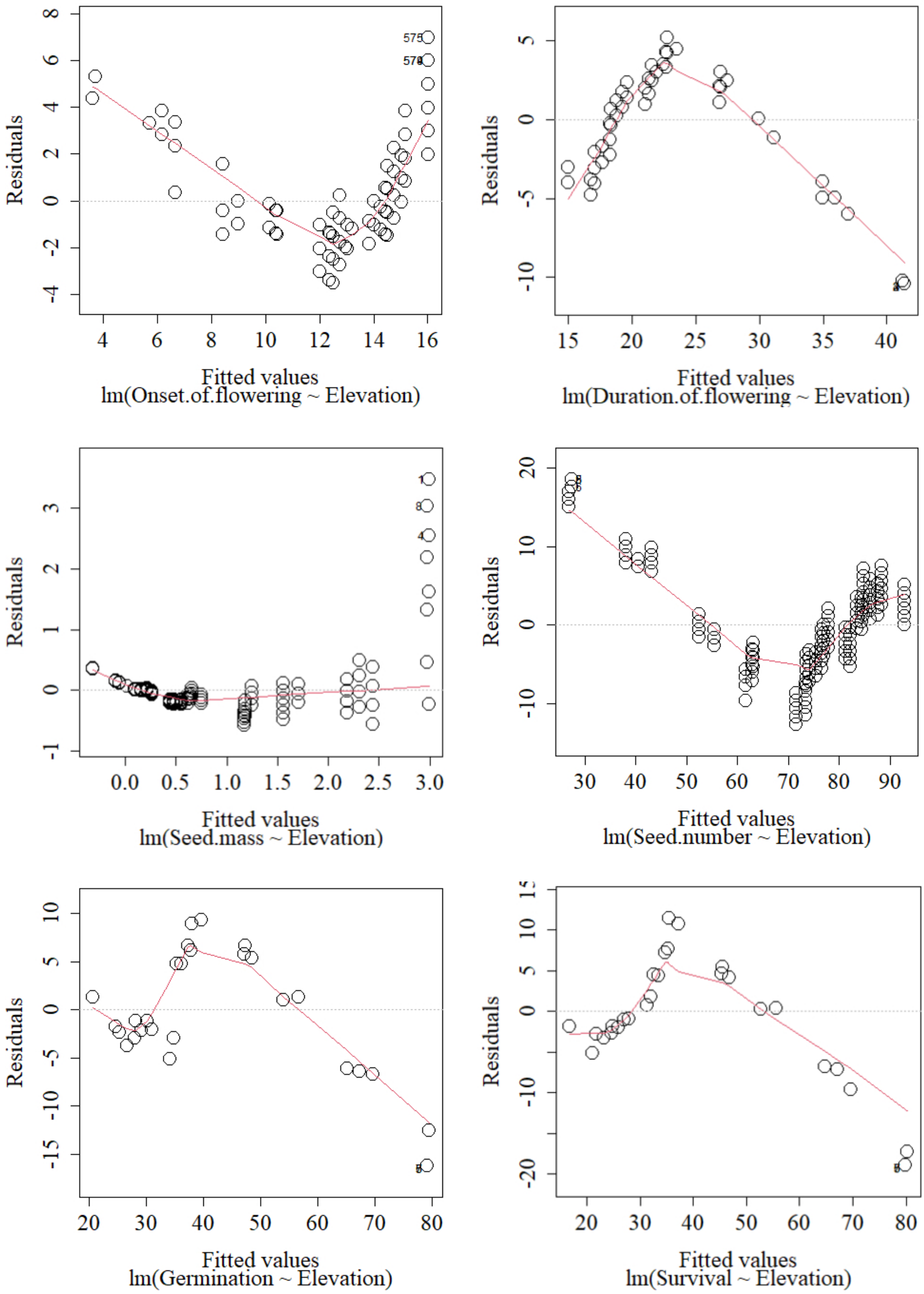


aov(Germination ~ Vegetation.zone)

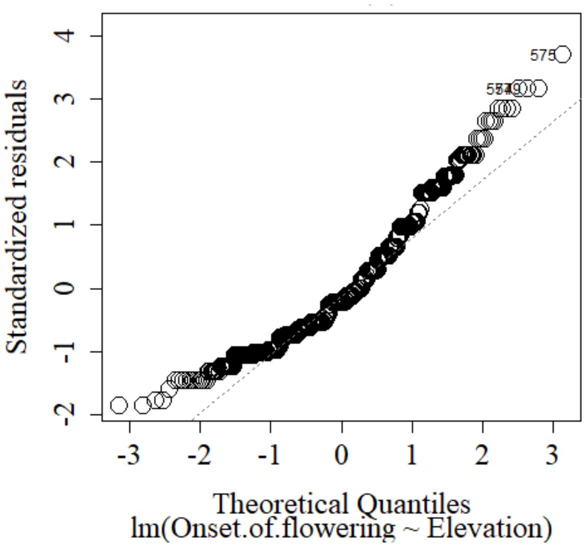


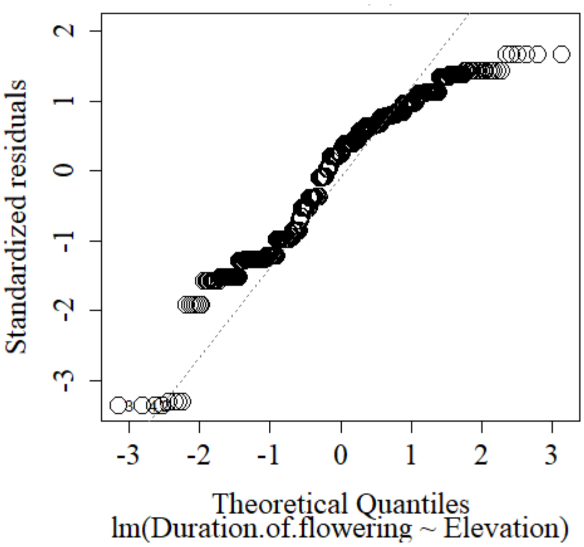
aov(Survival ~ Vegetation.zone)

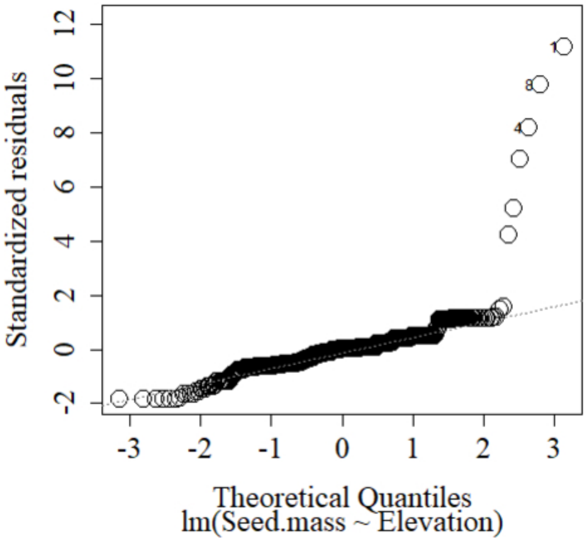
Appendix 3: Residuals versus Fitted Values Plots Demonstrating Model Convergence Between the Fitness Traits of *Arabis alpina* and Elevation

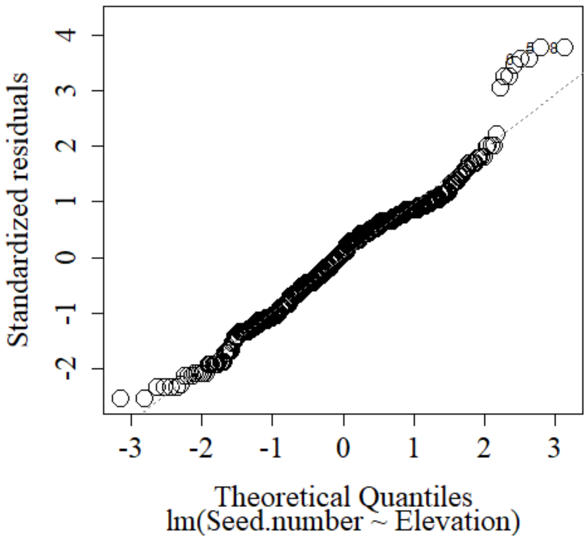


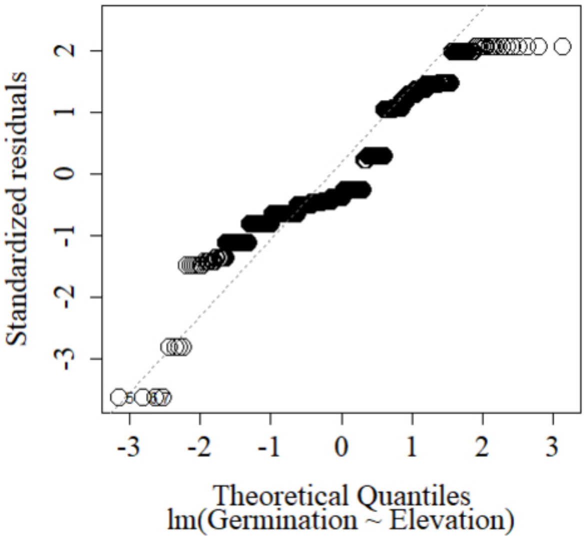
Appendix 4: Q-Q Plots of Standardized Residuals Demonstrating Model Convergence Between the Fitness Traits of *Arabis alpina* and Elevation

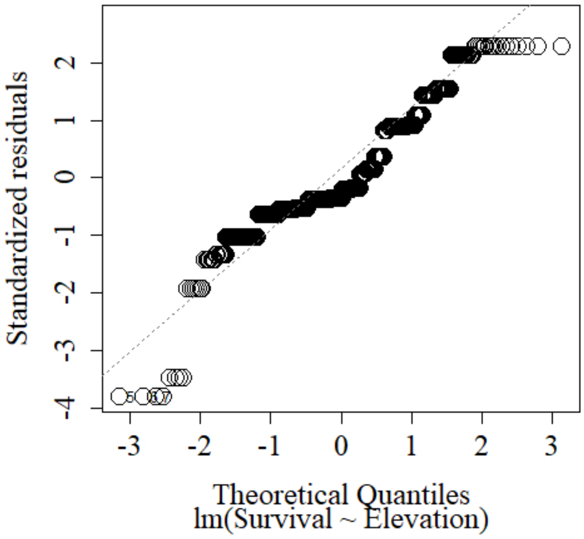




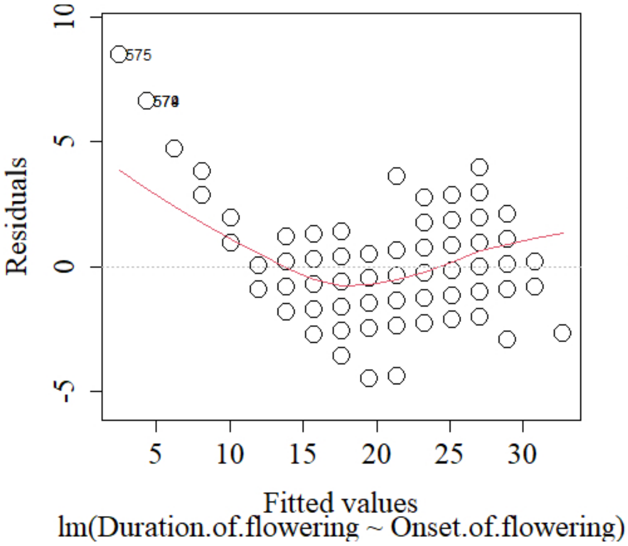


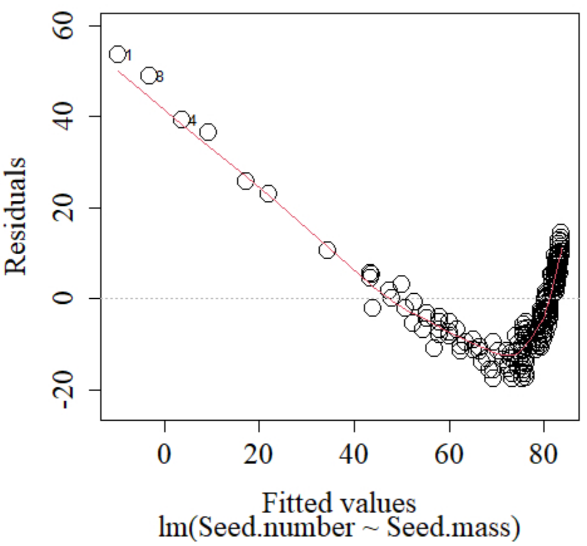


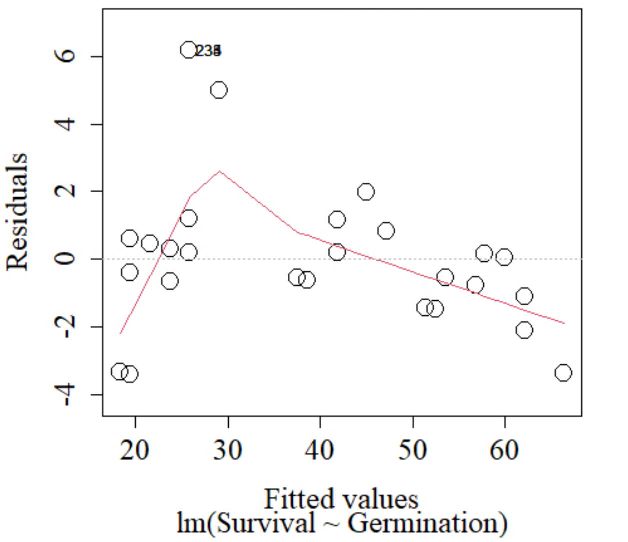


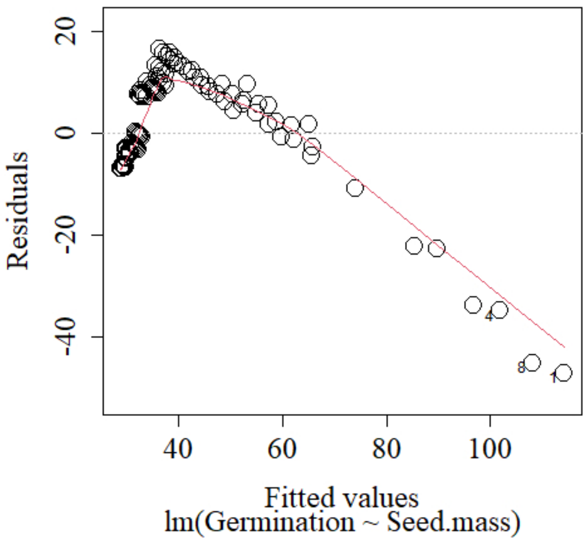


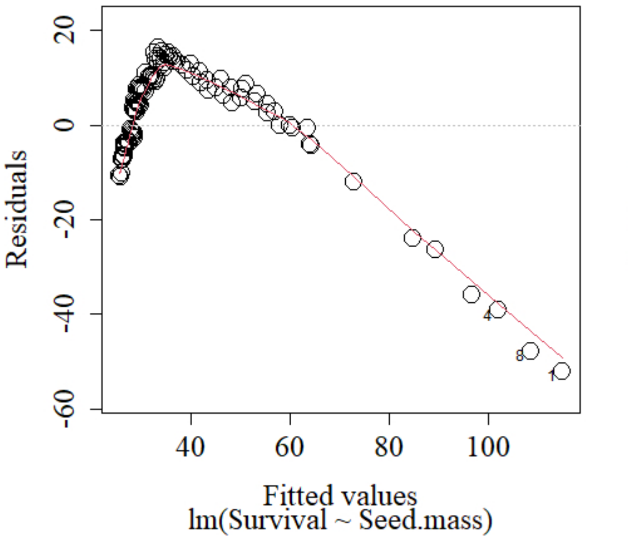
Appendix 5: Residuals versus Fitted Values Plots Demonstrating Model Convergence Between *Arabis alpina* Reproductive Fitness Traits

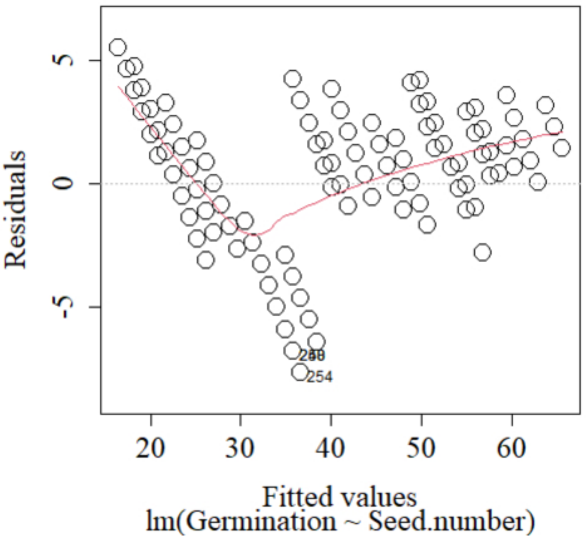


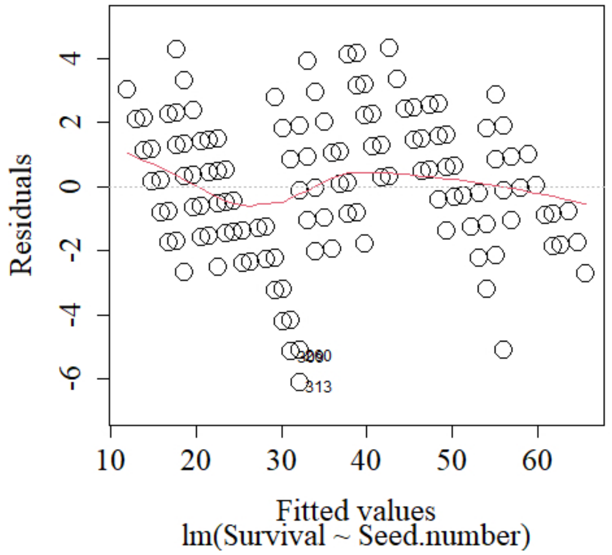




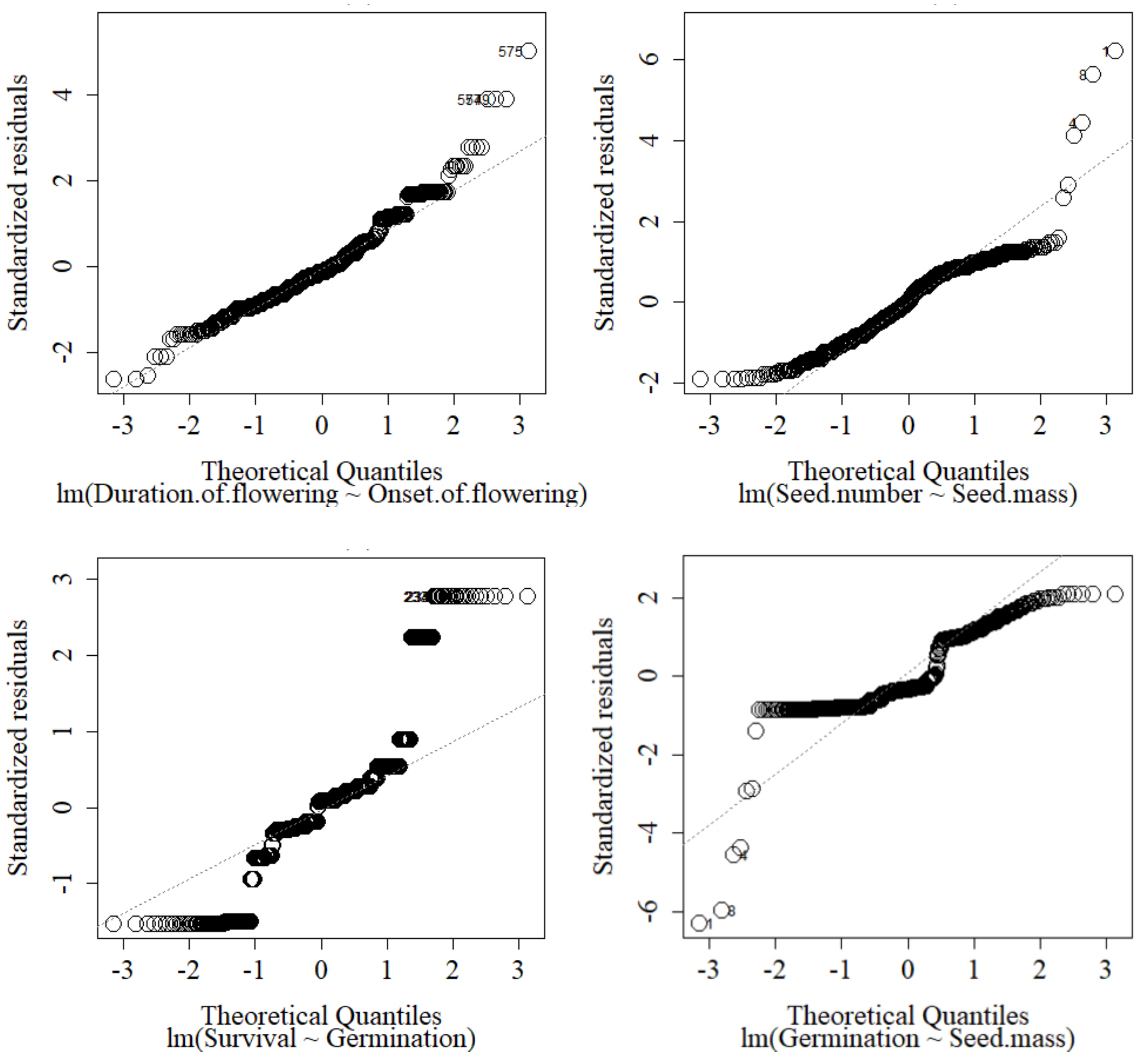


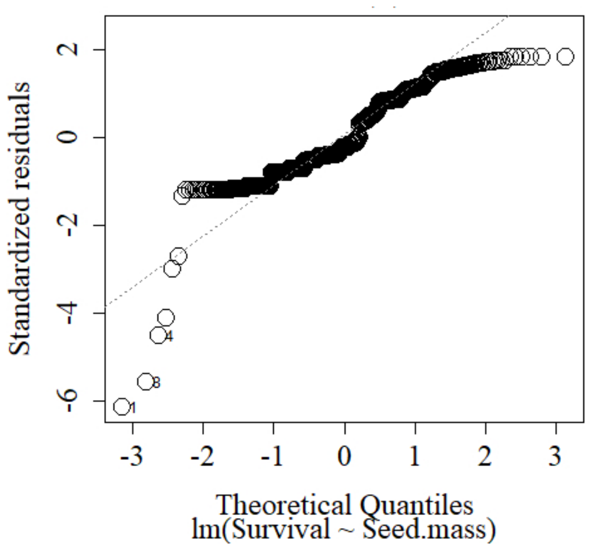


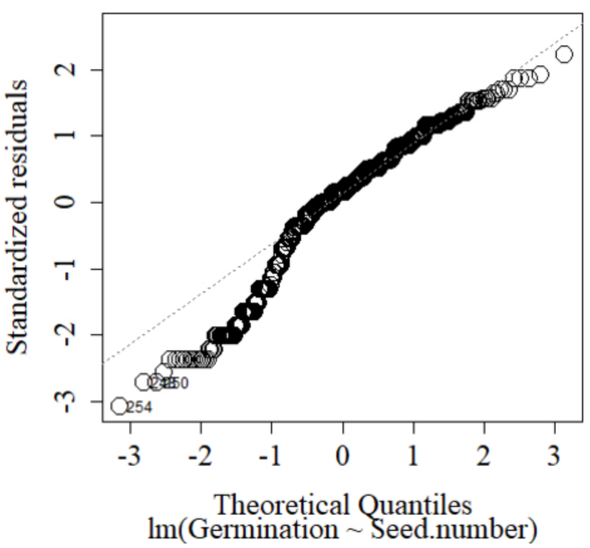


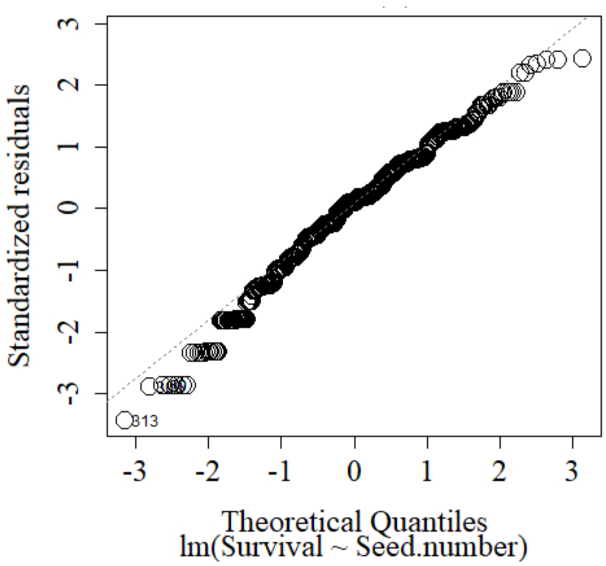


Appendix 6: Q-Q Plots of Standardized Residuals Demonstrating Model Convergence Between *Arabis alpina* Reproductive Fitness Traits




Standardized residuals
2
0
-2
-4
-6
1
8
4
-3
-2
-1
0
1
2
3
Theoretical Quantiles
lm(Survival ~ Seed.mass)


Standardized residuals
2
1
0
-1
-2
-3
254
250
-3
-2
-1
0
1
2
3
Theoretical Quantiles
lm(Germination ~ Seed.number)


Standardized residuals
3
2
1
0
-1
-2
-3
813
-3
-2
-1
0
1
2
3
Theoretical Quantiles
lm(Survival ~ Seed.number)